Vitamin D status among surgical orthopaedic patients in a private hospital in Johannesburg, South Africa

by
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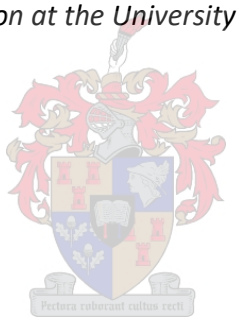

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DECLARATION

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ABSTRACT

Introduction: Vitamin D is a fat-soluble vitamin, classified as 9.10-secosteroid hormone precursor. Current research has revealed that optimal vitamin D status may be essential for a range of skeletal and non-skeletal outcomes. Vitamin D status was defined as sufficient (>30ng/mL), insufficient (20–30ng/mL) and deficient (<20ng/mL) for the purpose of this study. Data on 25(OH)D in South African adults is limited and vitamin D status assessment in the pre-operative adult orthopaedic patient has not yet been done. This study aimed to determine the vitamin D status of orthopaedic surgical patients and the impact of the surgical intervention on vitamin D status after the recovery period, as well as to determine the relationship between vitamin D status and demographic parameters, anthropometrics, sun exposure and vitamin D intake.

Methods: During the study period (April 2014–September 2014) patients were identified from orthopaedic surgical lists at Mulbarton Hospital, Johannesburg. Pre-operatively anthropometric measurements and relevant questionnaires (demographic, skin classification, sun exposure (calculated sun index) and short food questionnaire for vitamin D) were completed. Biochemical analysis of vitamin D was done pre-operatively. At a follow-up screening, vitamin D status, sun exposure and questionnaire for vitamin D intake were repeated.

Results: A total of 67 patients (67.2% females) were included. Average age was 50.9 ±12.3 years. Mean BMI of males and females was 30.5kg/m² and 31.7kg/m² respectively. The majority of patients had white skin (67.2%) and the rest brown to dark brown skin (32.8%). The median calculated sun index pre-operatively and post-operatively was 0.715; this can be calculated as 38 minutes sun exposure three times per week on arms and legs. Vitamin D oral intake from food was 195IU/day, and with supplementation added, 202IU/day. More than 65% of the population had insufficient plasma vitamin D status of which 29.9% were deficient with a mean value of 26ng/mL(SD 9.6).Correlation of vitamin D status with gender, smoking, vitamin D oral intake and BMI was not statistically significant. There was a weak positive correlation (r=0.249) between age and vitamin D status. There was a significant difference in vitamin D status of white and brown skin tones (9.82ng/mL)(p=0.025). There was a moderate positive correlation between vitamin D status and calculated sun index (r=0.451)(p=0.017). Sun exposure can be seen as a positive predictive indicator of vitamin D status where a calculated sun index of 1.159 yield a sensitivity of 68.2% and specificity of 56.5% (p=0.054). There was no statistical

significant change in sun exposure, vitamin D intake and vitamin D status after surgical intervention.

Conclusion: Even with sun exposure higher than the current recommendation, 25(OH)D levels were still very low; thus it can be suggested that the current recommendation for sun exposure might need to be doubled to achieve sufficient levels of 25(OH)D. Minor orthopaedic surgical interventions do not negatively influence sun exposure habits, vitamin D intake and thus vitamin D status, but these patients might be considered to be an “at risk” group as their mean BMI classification is obese and elective orthopaedic surgery is indicated.

ABSTRAK

Inleiding: Vitamien D is 'n vetoplosbare vitamien, geklassifiseer as 9,10-secosteroid hormoon voorloper. Huidige navorsing het aan die lig gebring dat optimale vitamien D status noodsaaklik is vir 'n verskeidenheid van skeletale en nie-skeletale uitkomste. Vitamien D status word gedefinieer as voldoende (>30ng/ml), onvoldoende (20–30ng/ml) en gebrekkig (<20ng/ml) vir die doel van hierdie studie. Data oor 25(OH)D in Suid-Afrika is beperk en status van volwasse ortopediese pre-operatiewe pasiënte nog nie bepaal nie. Hierdie studie het ten doel gehad om die vitamien D status van ortopediese chirurgie pasiënte te bepaal, die impak van die chirurgiese ingreep op vitamien D status ná intervensie asook die verhouding tussen vitamien D status en demografiese parameters, antropometrie, son blootstelling en vitamien D inname

Metodes: Gedurende die studietydperk (April 2014–September 2014) was pasiënte geïdentifiseer vanaf die ortopediese chirurgiese lyste by Mulbarton hospitaal in Johannesburg. Pre-operatiewe antropometriese metings en relevante vraelyste (demografies, vel klassifikasie, sonblootstelling (berekende sonindeks) en ‘n voedsel vraelys vir vitamien D) is voltooi. Biochemiese ontleding van vitamien D is pre-operatief gedoen. Met die opvolg besoek is vitamien D status, sonblootstelling en vitamien D voedsel vraelys herhaal.

Resultate: 'n Totaal van 67 pasiënte (67.2% vroue) was ingesluit. Gemiddelde ouderdom was 50.9 ±12.3 jaar. Gemiddelde liggaamsmassa-indeks (LMI) van mans en vrouens was 30.5kg/m² en 31.7kg/m² onderskeidelik. Die meerderheid van pasiënte het ‘n wit (67.2%) en die res ‘n bruin tot donkerbruin velkeur (32.8%) gehad. Die mediaan berekende sonindeks pre-operatief en post-operatief was 0.715; dit kan bereken word as 38 minute sonblootstelling drie keer per week op arms en bene. Gemiddelde orale inname van vitamien D vanaf voedsel was 195IU/dag, en met aanvullings 202IU/dag. Meer as 65% van die studiegroep het onvoldoende plasma vitamien D status gehad waarvan 29.9% gebrekkig was met 'n gemiddelde waarde van 26ng/ml(SD 9.6). Korrelasie tussen vitamien D status en geslag, rook gewoontes, orale vitamien D inname en LMI was nie statisties betekenisvol nie. Daar was 'n swak positiewe korrelasie (r=0.249) tussen ouderdom en vitamien D status. Daar was 'n beduidende verskil in 25(OH)D van respondente met ‘n wit en bruin velkleur (9.82ng/ml)(p=0.025). Daar was 'n matige positiewe korrelasie tussen 25(OH)D en berekende sonindeks (r=0.451) (p=0.017). Sonblootstelling kan gesien word as 'n positiewe aanwyser van vitamien D status. 'n Berekende sonindeks van 1.159 lewer

‘n sensitiwiteit van 68.2% en ‘n spesifisiteit van 56.5% (p=0.054) as ‘n aanwyser van voldoende vitamien D status. Daar was geen statisties beduidende verandering in sonblootstelling, vitamien D inname en vitamien D status na die chirurgiese ingryping nie.

Gevolgtrekking: Selfs met sonblootstelling hoër as die huidige kliniese aanbeveling, was vitamien D vlakke van hierdie populasie steeds baie laag. Daar kan voorgestel word dat die huidige aanbeveling vir sonblootstelling verdubbel word om voldoende vitamien D status te bewerkstellig. Klein ortopediese chirurgiese ingrepe beinvloed nie sonblootstelling, vitamien D inname en dus vitamien D status post-operatief negatief nie. Hierdie pasiënte kan as ‘n "risiko" groep oorweeg word as gevolg van hul gemiddelde LMI klassifisering as vetsugtig en elektiewe ortopediese chirurgie wat aangedui is.

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CONTRIBUTION BY PRINCIPAL RESEARCHER AND FELLOW RESEARCHERS

The principal researcher, Ronelle T. Vermaak, developed the idea and the protocol for the research project. The principal researcher planned the study, undertook all data collection and captured the data for analysis. The data was analysed with the assistance of a statistician, Ms Tonya Esterhuizen. The principal researcher interpreted the data and drafted the thesis. The supervisors, Prof. Renée Blaauw and Mrs Janicke Visser, provided input at all stages of the project and revised the protocol and thesis.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ADDENDA

LIST OF ABBREVIATIONS AND ACRONYMS

$1,25(OH)_2D$	1,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
7-DHC	7-dehydrocholesterol
AI	Adequate Intake
AIDS	Acquired Immune Deficiency Syndrome
BCC	Basal Cell Carcinoma
BMD	Bone Mineral Density
BMI	Body Mass Index
BSA	Body Surface Area
CANSA	Cancer Association of South Africa
CI	Confidence Interval
CM	Cutaneous Melanoma
CVD	Cardiovascular Disease
DALY	Disability-Adjusted Life Year
DBP	Vitamin D Binding Protein
DRI	Dietary Reference Intake
EAR	Estimated Average Requirements
ESPG	Endocrine Society Practical Guidelines
FGF-23	Fibroblast Growth Factor 23
HAART	Highly Active Antiretroviral Therapy
HMD	Health and Medicine Division
HPLC	High Performance/Pressure Liquid Chromatography
IOM	Institute of Medicine
IU	International Unit
LC-MS/MS	Liquid Chromatography–Tandem Mass Spectrometry
LOAEL	Lowest Observed Adverse Effect Level
MCC	Medicines Control Council
MED	Minimal Erythemal Dose
MRC	Medical Research Council
MS	Multiple Sclerosis
NOAEL	No Observed Adverse Effect Level
NRV	Nutrient Reference Value

PHF	Proximal Humerus Fracture
PTH	Parathyroid Hormone
RCT	Randomised Control Trial
RDA	Recommended Dietary Allowance
RNI	Reference Nutrient Intake
ROC	Receiver Operating Characteristic
RR	Relative Risk
RYGBP	Roux-en-Y gastric bypass surgery
SACN	Scientific Advisory Committee on Nutrition
SANHANES-1	First South African National Health and Nutrition Examination Survey
SCC	Squamous Cell Carcinoma
SD	Standard Deviation
SPF	Sun Protection Factor
SST	Serum Separator Tube
UK	United Kingdom
UL	Tolerable Upper Intake Level
UV	Ultraviolet
UVA	Ultraviolet A
UVB	Ultraviolet B
UVR	Ultraviolet Radiation
VDR	Vitamin D Receptor
Vitamin D_2	Calciferol or Ergocalciferol
Vitamin D_3	Cholechalciferol
WHI	Women's Health Initiative
WHO	World Health Organization

LIST OF DEFINITIONS

Vitamin D: A fat-soluble vitamin chemically related to the steroids and essential for the normal formation of bones and teeth and for the absorption of calcium and phosphorus from the GI tract. Ultraviolet rays activate a form of cholesterol in an oil of the skin that is converted to a form of the vitamin in the kidney.[1]

Vitamin D_2 (Ergocalciferol): A fat-soluble, crystalline unsaturated alcohol produced by ultraviolet irradiation of ergosterol in plants. It is used as a dietary supplement in the prophylaxis and treatment of rickets, osteomalacia, and other hypocalcaemic disorders. Also called oleovitamin D_2.[1]

Vitamin D_3 (Cholecalciferol): An antirachitic, white, odourless crystalline unsaturated alcohol that is the predominant form of vitamin D of animal origin. It is found in most fish-liver oils, butter, brain, and egg yolk and is formed in the skin, fur, and feathers of animals and birds exposed to sunlight or ultraviolet rays. Also called activated 7-dehydrocholesterol.[1]

Vitamin deficiency: A state or condition resulting from the lack of or inability to use one or more vitamins. The symptoms and manifestations of each deficiency vary, depending on the specific function of the vitamin in promoting growth and development and maintaining body health.[1]

25(OH)D: (25-hydroxyvitamin D) also known as calcifediol or calcidiol: a major circulating metabolite of vitamin D.[1]

Ultraviolet: Indicating electromagnetic radiation of wavelength shorter than that of the violet end of the spectrum, with wavelengths of 4–400 nm (nanometers). Ultraviolet A (UVA) is electromagnetic radiation with wavelengths between 320–400nm, containing over 99 percent of radiation that reaches the surface of the earth. Ultraviolet B (UVB) is electromagnetic radiation with wavelengths between 290–315nm, containing less than 1 percent of the ultraviolet radiation that reaches the earth's surface. Ultraviolet B causes vitamin D production and sunburn.[1]

CHAPTER 1: LITERATURE OVERVIEW

1.1 Introduction

The tale of the compound, calciferol, is a remarkable but well-known one regarding a prohormone that is involved in calcium and phosphorus homeostasis and bone mineralisation.[2,3,5,6] Vitamin D, also known as the "sunshine vitamin",[7-10] is often taken for granted as it is believed to be abundant in a healthy diet and sunny environment.[2] In the last decade the interest in vitamin D among scientists has escalated significantly as scientists have suggested that the effect of vitamin D continues beyond its function in healthy bones. There has also been a plethora of research and publications linking vitamin D insufficiency and deficiency with an increasing number of medical conditions.[10] Vitamin D relates to both ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3). Cholecalciferol is more biologically active and the preferred form of vitamin D, but both are used in nutritional supplements and food fortification.[11] Only a few foods naturally contain vitamin D and in South Africa only a limited number of foods are fortified[2] with vitamin D, mostly cereal and margarine.[12]

Vitamin D status varies significantly between different countries in Asia, Europe, the Middle East and Africa. This is the result of different degrees of sun exposure, clothing, pollution, use of sunscreen, latitude, altitude, season, ethnic group, skin pigmentation,[1,7,13] genetics, body mass index (BMI), age,[1] dietary intake, fortification programmes and supplementation use.[1,13]

Vitamin D deficiency is fast being acknowledged as one of the most widespread medical conditions in the world.[3,9,14,15] Rickets has been described as the "tip of the Vitamin D-deficiency iceberg".[16] Vitamin D insufficiency and deficiency not only contributes to metabolic bone disease in children and in adults, but it may also increase the risk of many other common chronic diseases.[2] Some of the areas of interest for researchers are autoimmune diseases, cardiovascular disease, cancer,[2,3] multiple sclerosis, rheumatoid arthritis, type I diabetes, and orthopaedic patients, to name but a few.[2,16]

To the best knowledge of the author, the vitamin D status of South African adult patients in private hospitals that had elective orthopaedic surgery has not yet been determined.

1.2 History and Current Situation

From an evolutionary viewpoint, vitamin D was formed from sunlight exposure in phytoplankton and zooplankton present in the oceans for more than 500 million years.[2,17,18] It was suggested very early (1924, 1927) that the vitamin D found in oily fish was due to the dietary intake of the vitamin from these plankton. The seasonal variations in the vitamin D content of oily fish correlate with higher sun exposure in summer and lower exposure in winter months.[17]

In terms of human history, the very beginning of the industrial revolution in Northern Europe was where the vitamin D deficiency epidemic originated.[2,17,19] People began gathering together in cities and lived in dwellings that were built very close to one another. Burning of wood and coal caused air pollution and children living in these developed areas had very little direct exposure to sun.[2,17] The first clinical description was in 1651 by Glisson, De Boot and Whistler, describing children with rickets (growth retardation and skeletal deformities, including rachitic rosary (bony projections along the rib cage) and either knock knees or bow legs).[2,19] The disease migrated to the northern United States (New York and Boston), and to Leiden in the Netherlands. By the 1900s, rickets was so devastating and widespread that approximately 80–90% of children suffered from the disease. The first Caesarean section under modern antiseptic conditions (1888) was performed in Glasgow by Murdoch Cameron (31 March 1847–28 April 1930) to assist a young rachitic woman (Catherine Colquhoun) in birthing because the baby could not be delivered naturally owing to her deformed, flattened pelvis.[20] In such rachitic women, birthing by Caesarean section became very common.[17] The seldom-used procedure that usually culminated in the death of the mother became a safe and routine operation under antiseptic conditions.[20]

The first recognised association between sunshine and rickets was made in the beginning of the 19th century in 1822 by Jędrzej Śniadecki (1768–1838).[6] He published his clinical annotations of children living in the inner city in Warsaw with a high occurrence of the disease versus the children in the rural areas outside Warsaw that did not have the disease. This was followed by observations by Palm about children living in industrialised cities in Great Britain that were at high risk of developing rickets. His colleagues from China and India wrote that malnourished children in these countries that lived in squalor were actually free of this disease. Palm came to the conclusion that sunbathing could

prevent rickets and that a type of sunshine recorder needed to be developed to measure the bone-healing properties of the sun. This fell on deaf ears until 1919, when Huldschinsky reported that exposure to ultraviolet radiation from a mercury arc lamp resulted in the cure of rickets.[2,3,17-19] Hess and Unger (1921) noted effective treatment for rickets when children were exposed to sunlight over a period of several months on the roof of a hospital in New York.[1] Between 1922 and 1924, Hess and Weinstock[21] and Steenbock and Black[22] published data in *The Journal of Biological Chemistry* about irradiating a wide variety of substances such as vegetable oils and grasses. They reported that the irradiation process gave anti-rachitic activity to these foods. It was also shown that sun exposure prevented rickets in rats. At about the same time Elmer V McCollum at the University of Wisconsin and later at Johns Hopkins University designed an ingenious series of experiments where he destroyed the vitamin A in cod liver oil and identified the remaining separate antirachitic substance. In the 1922 publication of these experiments, the designation of vitamins in alphabetic order was followed and vitamin D was named, as vitamin B and C had been recently named.[23] Steenbock and Black, and Hess and Weinstock, as two independent research groups, experimented on irradiation of skin and introduced the model that the irradiation of food with ultraviolet (UV) radiation would prevent and treat rickets, to a large extent the same as cod liver oil did. This ultimately led to the fortification of milk and other products, and this fortification process in essence eradicated rickets.[2,17,23] Fortification with vitamin D became so popular in the 1930s and 1940s that peanut butter, bread, hot dogs and soda were fortified. Schlitz Brewery introduced fortified beer[2,17] and marketed it as beer with “sunny energy in both summer and winter”.[2]

By 1925 Adolf Windhaus from Germany was considered the leading expert in sterol research. He was awarded the Nobel Prize for Chemistry in 1928. In partnership with other scientists, such as Hess and Rosenheim, the teams deduced that ergosterol was the probable parent substance of vitamin D that was found in food. Later, back in Germany, Windhaus isolated three forms of vitamin D and called them D_1 and D_2 (from plant sterols) and D_3 (from irradiated skin).[23] The British team of scientists led by FA Askew successfully defined the chemical makeup of D_2 in 1931; this is the form of vitamin D found in irradiated food (now called ergocalciferol). Only five years later in 1936 the molecule 7-dehydrocholesterol was synthesised and then converted through irradiation to D_3 (now known as cholecalciferol) by Windhaus.[10,23] Over the next 40 years research teams tried to map the metabolic pathway of vitamin D. Scientists did not possess all the tools until the

1960s, when new techniques using radioactive-labelled substances emerged. Between 1968 and 1971 great progress was made in the metabolic processing of vitamin D. In 1968 the team of DeLuca isolated an active vitamin D metabolite that was identified as 25-hydroxyvitamin D_3 and later demonstrated to be produced in the liver. During the next two years it was shown that there was a second metabolite produced in the kidney. In 1971 three research groups published papers reporting the chemical structure of this metabolite that was identified as 1.25-dihydroxyvitamin D_3. It was also around this time that the link with calcium metabolism was finally established.[10] In the 1980s, evidence was found by several groups of researchers that vitamin D was present in other cells that were not part of calcium metabolism, such as brain, lymphocytes, skin and malignant tissues. The immunosuppressant function of vitamin D was used in research on autoimmune disease. In 1994 the US Food and Drug Administration approved a topical calcitriol treatment for psoriasis.[10,23] Now as we are entering the twenty-first century, researchers will keep on pursuing new applications for vitamin D while remembering the important role it plays in bone health.[23]

1.3 Definition of Vitamin D Status

The Endocrine Society is the largest global membership organisation that represents professionals from the field of endocrinology. The society has members from 122 countries, with its headquarters in Washington DC.[77] On March 15, 2016, the Institute of Medicine (IOM), a division of the National Academies of Sciences, Engineering, and Medicine (the National Academies) that focuses on health and medicine, was renamed the Health and Medicine Division (HMD). The National Academies are private, non-profit American institutions that provide independent, objective analysis and advice related to science, technology and medicine.[78] Note that all reports issued by the new HMD division will be cited as reports of the National Academies of Sciences, Engineering, and Medicine. Reports issued prior to June 30, 2015 will continue to be cited as IOM reports in perpetuity.[78]

The reference values for the different categories of vitamin D status are indicated in Table 1.2. It should be noted that there are two schools of thought in defining vitamin D status; first there is the Institute of Medicine (IOM) 2011 report on dietary intakes for calcium and Vitamin D,[79,80] and secondly the Endocrine Society Clinical Practice Guidelines on

Evaluation, Treatment, and Prevention of Vitamin D Deficiency.[37] To date (2016) the IOM has not released newer guidelines.[80]

The objective of the Endocrine Society Clinical Practice Guidelines on Evaluation, Treatment, and Prevention of Vitamin D Deficiency is "to provide guidelines to clinicians for the evaluation, treatment, and prevention of vitamin D deficiency with an emphasis on the care of patients who are at risk for deficiency".[37,81] The IOM Committee, focused on a healthier population, concluded "that available scientific evidence supports a key role of calcium and vitamin D in skeletal health, consistent with a cause-and-effect relationship and providing a sound basis for determination of intake requirements".[79,81] The IOM concluded that after careful deliberation the evidence showed that bone health was the only outcome that satisfied the criteria for vitamin D recommendations as an "indicator" for healthy skeletal outcomes.[37]

There are several main positions of discrepancy between the two published recommendations. The first main point is the sufficient vitamin D level; IOM states >20ng/mL (>50nmol/L).[18,79,80,82,83] and the Endocrine Society recommends levels of >30ng/mL (>75nmol/L).[18,37,82,84] The IOM also concluded that evidence was inconsistent and inconclusive regarding nonskeletal benefits and that no recommendations could be made in this regard.[79,80] The Endocrine Society takes a more futuristic view in stating that "recommendations will likely need to be revised as future evidence accumulates".[37]

The 2016 report on vitamin D and health of the Scientific Advisory Committee on Nutrition (SACN) states that the United Kingdom (UK) currently defines the lower limit of adequacy at 10ng/mL (25nmol/L) based on the increased risk of osteomalacia and rickets at concentrations below this level.[85] It is recommended by SACN that the serum 25(OH)D levels of all individuals in the UK should not fall below 10ng/mL (25nmol/L) at any time of the year.[85] The SACN advises on nutrition and related matters to Public Health England and other UK government organisations.

In a recent editorial in The New England Journal of Medicine Manson et al warns against the misapplication and misinterpretation of the IOM reference values of vitamin D as such misunderstandings can lead to unnecessary vitamin D screening and supplementation that can lead to mounting health costs. Clinical judgement and individualized interventions is recommended to avoid overscreening and overprescribing of vitamin D supplements.[137]

Reid concludes in a recent published article that individuals should not have 25(OH)D levels under 16ng/mL since such a strategy would prevent osteomalacia, a condition usually seen with levels under 10ng/mL. The author adds that osteomalacia and rickets are the only conditions where abundant clinical evidence is available with not enough conclusive trial evidence of other beneficial effects of vitamin D.[138]

Table 1.1: Definition of Vitamin D Status

Vitamin D Status	Plasma Concentrations					
	Endocrine Society		IOM		SACN	
	ng/mL	nmol/L	ng/mL	nmol/L	ng/mL	nmol/mL
Deficient[2,3,6,8,9,14,16,18,37,50(IOM)]	<20	<50	<12	<30	<10	<25
Insufficient[3,6,9,14,16,18,34,37,54]	20–29	50–72.5	12-20	30-50		
Sufficient[2,3,8,9,14,18,24,34,35,37,86 (ESPG)]	30–60(-100[32])	75–150	>20* >16#	>50* >40#	>10	>25
Toxic[8,9,16,13,18]	>150	>374	>50	>125		

1ng/mL = 2.5nmol/L

IOM – Institute of Medicine

SACN - Scientific Advisory Committee on Nutrition

** Should cover ≥ 97.5% of the population*

 # Should cover approximately half the population

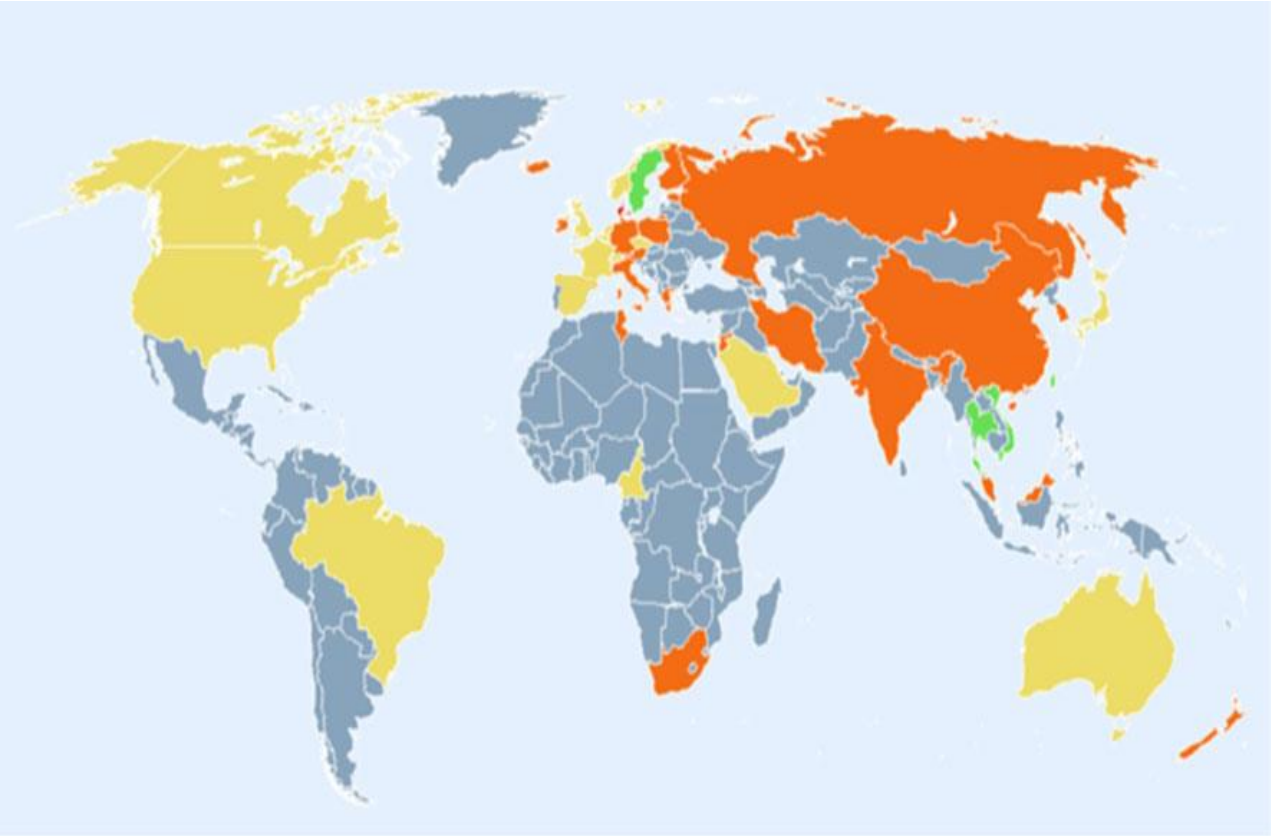
There are two motivations for positioning the sufficient range for 25(OH)D at >30 ng/mL: one proposes that levels of PTH increase when plasma levels of 25(OH)D fall beneath 30ng/mL[16,34,37] and the other proposes that active calcium absorption is best when the level of 25(OH)D is 30ng/mL.[34,37] Whereas secondary hyperparathyroidism can be prevented with 25(OH)D levels of at least 20ng/mL,[86] 25(OH)D should most likely be at least 30ng/mL to maximise cellular health[2,3,14,6,24,34,35,86] and uphold skeletal health.[37] Levels of at least 30ng/mL are necessary to start antifracture effectiveness.[37] Jesudason et al. concluded in their study on the relationship between serum 25(OH)D levels and bone resorption markers (hydroxyproline, pyridinoline and deoxypyridinoline) that these bone resorption markers can be detected when 25(OH)D levels fall beneath 24ng/mL; thus levels above 24ng/mL may be required for optimum bone health.[55]

1.4 Prevalence of Vitamin D Deficiency

Vitamin D status in various European countries, North America, Asia and Oceania has been assessed in numerous studies and in great detail.[13,16,18,24-26] In the analysis done by Wahl et al. on the global representation of vitamin D status, 200 studies from 46 countries were scrutinised and of these, 46 were considered representative. The largest number of studies came out of Europe (48%), followed by North America (27%) and Asia-Pacific (16.5%). These results were then summarised in a world map with colour coding that gives a snapshot of 25-hydroxyvitamin D (25(OH)D) levels around the globe (Figure 1.1).[26] The global prevalence of vitamin D deficiency and insufficiency varies from 3%–91%, but the interpretation of the data from different studies is hindered by the large variation in cut-off points used to define deficiency and insufficiency that can range from 5ng/mL to 30ng/mL.[10,18]

Adult vitamin D status studies in Africa[27] and specifically South Africa are few and far between – A study done in 1975 on elderly South African patients with hip fractures found mean serum 25(OH)D concentrations at 17.7ng/mL, SD 9.2ng/mL.[121] One study on black South African woman (*n*=43) after delivery of their babies done in 1987 in the Transkei found normal serum 25(OH)D levels of 32.7ng/mL.[121] A study done on vitamin D status of 10-year-old urban South African children (2011) concluded that the vitamin D status of the children was generally good, with only 7% deficient;[28] another study on vitamin D formation by sunlight in Johannesburg concluded that the seasonal variation in 25(OH)D was a consequence of increased clothing and less time spent outdoors during winter, rather than less UV radiation reaching the earth as seasonal changes in *in vitro* production were not found.[29] Haarburger et al. found in 2009 that the median value of their study population (age 2–64) was 19.3ng/mL (range 2.2–42.4ng/mL). Vitamin D deficiency, here defined as <18ng/mL, was found in 41% of subjects.[30] In a recent (2015) review of South African studies (25 studies) assessing 25(OH)D it was found that whites had the highest 25(OH)D (34.4ng/mL), followed by blacks (28.4ng/mL), mixed ancestry (20.4ng/mL) and Asian Indians (16ng/mL). According to this data it is apparent that in South Africa, Asian-Indians are vitamin D deficient, while blacks possibly have insufficient and whites sufficient vitamin D.[121] Results indicate that population groups in South Africa with moderate to dark skin pigmentation are at high risk of deficiency when UVB radiation is limited owing to seasonal fluctuations.[122]

A recent study done in Israel, an area characterised by mostly sunny year-round weather, revealed Vitamin D deficiency to be prevalent. In this study, 78% of subjects had insufficient vitamin D serum concentrations and 27% of these were also vitamin D deficient.[48]



>30ng/mL **20-29.9ng/mL** **10-19.9ng/mL** **<10ng/mL**

Permission to use the figure was obtained from the author (2015, October 15).

Figure 1.1: Mean 25(OH)D in Adults (>18 years) Around the World (2012)[26]

1.5 Metabolism of Vitamin D

Vitamin D is a fat-soluble vitamin,[31] but not a true vitamin (a substance that our body cannot manufacture and can only be obtained from dietary sources).[23] Vitamin D is correctly classified as a 9,10-secosteroid hormone precursor.[7,23,32] Vitamin D is a secosteroid hormone because one of the rings of its structure has a broken carbon-carbonbond (this occurs in the 9,10 carbon-carbon bond).[32] A hormone can be described as a chemical substance that is produced in one organ and then transported to the target

organ. Vitamin D is naturally present in only a few foods, added to some food and available as a dietary supplement. Vitamin D is also produced endogenously when skin is exposed to ultraviolet rays from sunlight.[31] Vitamin D refers to ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3)[11] (Figure 1.2).[18,32] When exposed to solar ultraviolet B radiation (UVB, 290-315nm),[6,9,17,33] 7-dehydrocholesterol (7-DHC) in the skin absorbs the UVB radiation and is transformed to pre-vitamin D_3, which is immediately transformed to vitamin D_3 in a heat-dependent process.[2,6,9,11,33-35] During the conversion process, vitamin D_3 is expelled from the plasma membrane into the extracellular space. The vitamin D binding protein in the dermal capillary bed has an affinity for D_3 and pulls it into circulation.

Vitamin D_2 (Ergocalciferol) Vitamin D_3 (Cholecalciferol)

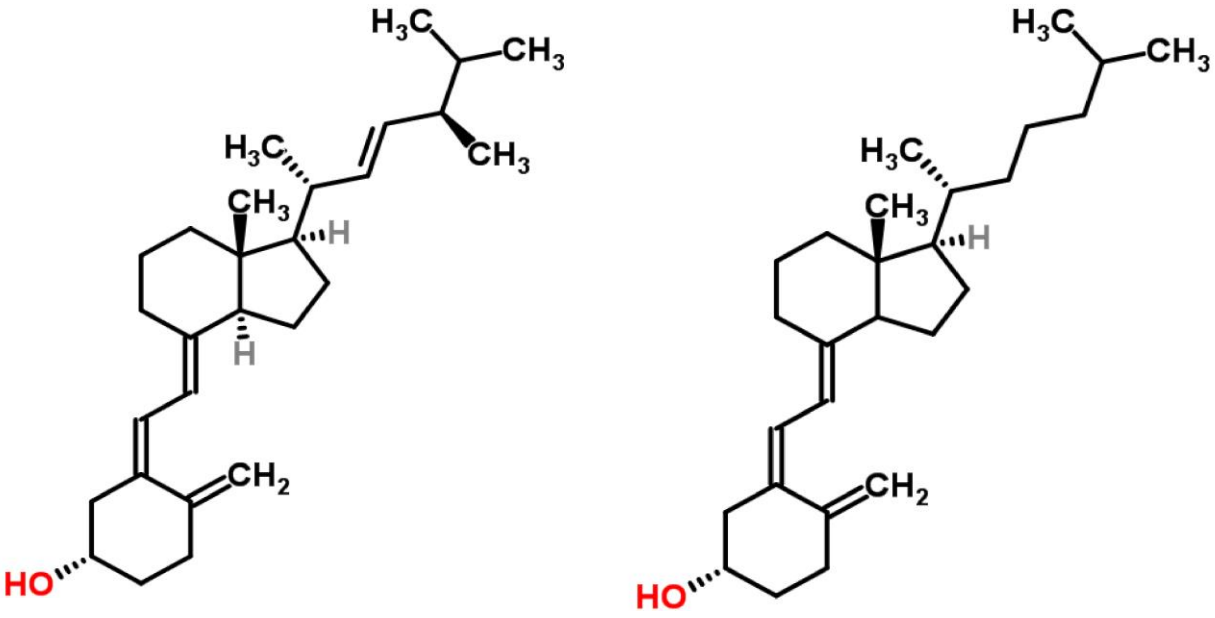


Figure 1.2: Chemical Structure of Vitamin D_2 and Vitamin D_3[18]

Vitamin D from dietary sources is bound to the vitamin D binding protein (DBP),[6,34] taken up by enterocytes and packaged into chylomicron remnants that enter the bloodstream via the lymphatic system[8] and transported to the liver or fat for storage. In the liver it is transformed to 25-hydroxyvitamin D (25(OH)D), its main circulating form. In this structure it has restricted biological activity. In the kidney 25(OH)D is converted to its most active circulating metabolite, 1.25-hydroxyvitamin D (1,25$(OH)_2$D) by the enzyme CYP27B1 under the influence of parathyroid hormone (PTH)[2,6,16,18,34] and a multitude of other factors, including serum phosphorus, calcium and fibroblast growth factor 23 (FGF-23).[18]

CYP27B1 is also expressed extrarenally in a large number of tissues, including bone, prostate, placenta, T-lymphocytes, keratinocytes, macrophages, dentritic cells, several cancer cells and the parathyroid gland, and this enables the production of $1,25(OH)_2D$.[18] The prime action of $1,25(OH)_2D$ is maintaining calcium and phosphorus homeostasis and bone health;[6,11] this happens through the vitamin D receptor to improve intestinal calcium absorption and to encourage the maturation of osteoclasts[6] (Figure 1.3).[36]

In spite of this it has been progressively acknowledged that vitamin D has pleiotropic effects on an array of extraskeletal tissues, suggesting a vital role in disease prevention and health.[6]

Sun exposure over extended periods does not result in excess production of vitamin D_3 and thus intoxication, as the previtamin D_3 that is produced and the thermal isomerisation product - vitamin D_3 - does not get absorbed into the circulation and will absorb solar UV radiation and isomerise to numerous photoproducts that are believed to have little action on calcium metabolism.[2]

Anything that influences the number of solar UVB photons that infiltrate the skin or changes the amount of 7-DHC in the skin will influence the cutaneous production of vitamin D_3. The level of 7-DHC in the epidermis is reasonably constant until in later life, when it begins to decline. A person 70 years of age exposed to the same quantity of sunlight as a 20-year-old will produce approximately 25% of the vitamin D_3 that the 20-year-old individual can formulate.[2]

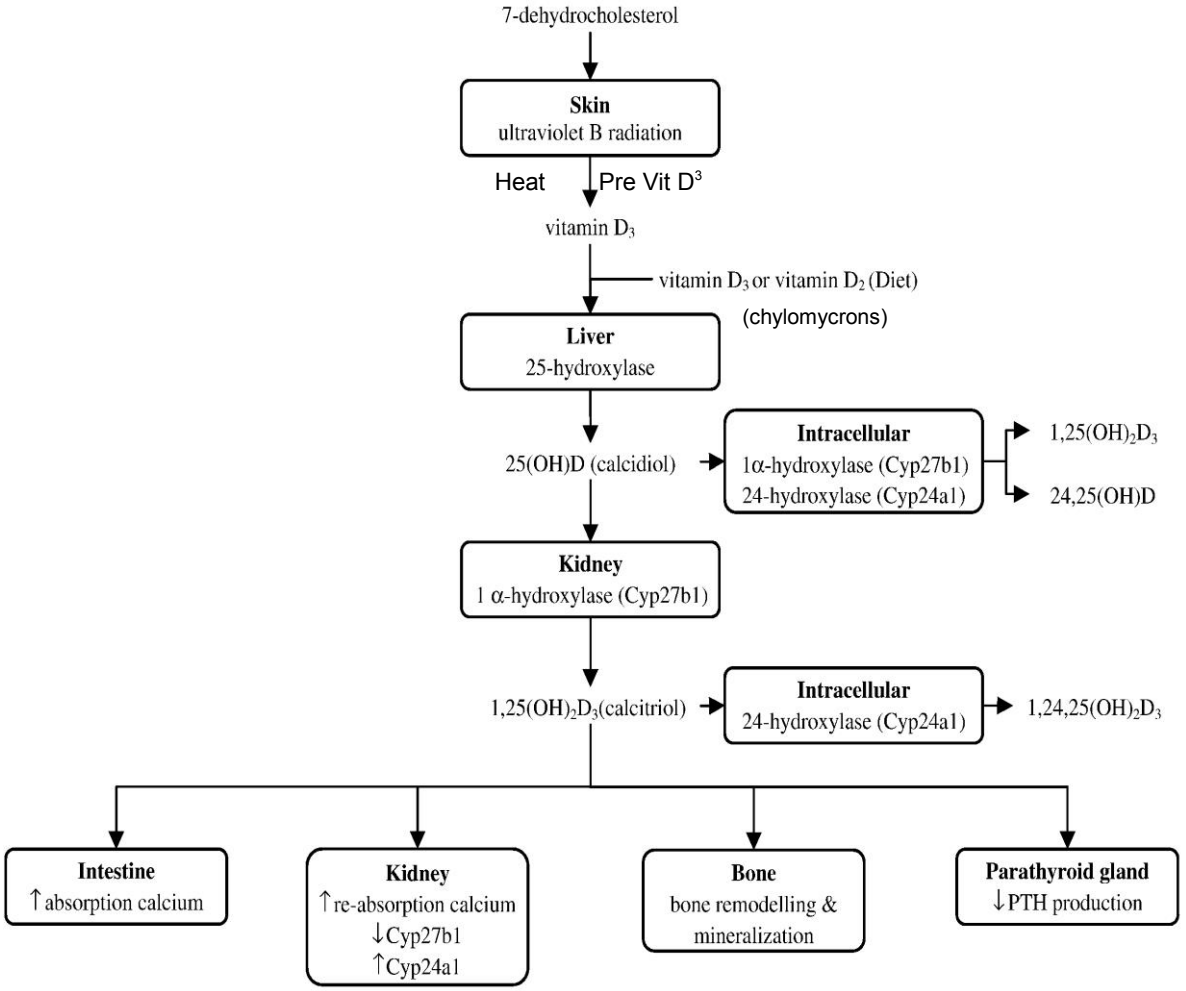


Figure 1.3: Flow Chart of Vitamin D Metabolism and its Function in Calcium and Skeletal Homeostasis[36]

1.6 Sources of Vitamin D

The sources of vitamin D include oral intake (from diet and/or supplements in the form of cholechalciferol [D_3] or ergocalciferol [D_2]) (Figure 1.2) and cutaneous synthesis from sunlight (in the form of cholechalciferol [D_3]).[6,9,17,19,24,33] Vitamin D supplements are covered in more detail in Section 1.12.

Vitamin D occurs naturally in very few foods (Table 1.1). Food sources of vitamin D are oily fish such as sardines (291 IU/100g), salmon (528 IU/100g), herring (1000 IU/100g) and kipper (1000 IU/100g), egg yolk (162 IU/egg) and cod liver oil (500 IU/ ml). In South

Africa, food sources that contain vitamin D are more limited than in other northern hemisphere countries. For example, irradiated mushrooms are a good source of vitamin D,[2,11] but in South Africa mushrooms are not exposed to UV light and contain no vitamin D.[12] Very few food items in South Africa are fortified with vitamin D; only certain cereals like Kellogg's® (e.g. Special K, Rice Crispies, Frosties, Corn Flakes and All Bran) (28 IU/25g) and FUTURELIFE® (300 IU/50g) are fortified; most other cereals contain no vitamin D.[12] According to the Medical Research Council of South Africa food composition tables, margarine contains between 10–12 IU/5g, depending on the brand used. Health bars like PVM Energy® bars contain 209 IU per bar and milk powders contain 100 IU/25g.[12] When comparing these figures with the recommended dietary allowance (RDA) of vitamin D for adults of the Institute of Medicine (IOM) of 600 IU/day,[32] it can be noted that very few foods make a considerable contribution to the daily vitamin D intake. FUTURELIFE® contains 50% of the RDA and Kellogg's® cereals only 5%.

Humans have evolved to derive most of their vitamin D requirements from cutaneous synthesis. Most people will get more that 90% of their vitamin D requirements from casual exposure to sunlight.[2] Vitamin D and sun exposure are covered in more detail in Section 1.11.

Table 1.2: Dietary Sources of Vitamin D in South Africa

Source	Portion	Amount	
		µg	IU
Natural Foods[12,38]			
Butterfish, grilled	100g	25.94	1037
Herring, grilled	100g	25.94	1037
Kipper, baked (smoked herring)	100g	25.00	1000
Salmon, canned, drained	100g	13.21	528
Cod liver oil	5ml	12.50	500
Pilchards, canned in brine / tomato sauce	100g	8.00	320
Tuna, canned in oil, drained	100g	7.40	296
Sardines, canned in oil, drained	100g	7.28	291
Sardines, canned in tomato sauce, drained	100g	6.79	271
Egg, whole (vitamin D in yolk)	51g	4.05	162
Liver, chicken	100g	1.79	72
Liver, beef	100g	1.55	62
Veal, cooked	100g	1.50	60
Pork, cooked	100g	1.00	40
Beef, cooked	100g	0.65	26
Fortified Foods[12,38]			
Health Bar PVM®	55g	5.23	290
Milk powder	25g (250ml milk)	2.50	100
FUTURELIFE® Smartbar®	40g	0.9	36
Margarine	14g	0.70	28
Certain cereals	30–50g	0.84–3.38	34–135

1.7 Vitamin D Status Assessment

Plasma 25-hydroxyvitamin D (25(OH)D) concentration is the parameter of choice for assessing vitamin D status because of its long half-life (approximately 2–3 weeks).[13,15,24,33,44] The whole-body half-life of radio-labelled vitamin D is approximately 2 months.[45] Seamans and Cashman concluded in their systematic review of 36 randomised control trials (RCTs) and four before/after studies, that circulating 25(OH)D is a reliable and robust marker of vitamin D status.[46]

Although 1,25 dihydroxyvitamin D (1,25$(OH)_2$D) is the biological active form of vitamin D, it is not considered appropriate to assess vitamin D status for various reasons. The half-life of circulating 1,25$(OH)_2$D is very short[2,6,30] (4–6 hours),[3,9] the circulating levels of 1,25$(OH)_2$D are a thousand-fold lower than those of 25(OH)D, and as a person becomes vitamin D deficient, there is diminished intestinal calcium absorption which will lower the ionised calcium momentarily. In the short term this signal is then recognised by the calcium sensor in the parathyroid glands to boost the secretion and production of parathyroid hormone (PTH) (secondary hyperparathyroidism). PTH regulates calcium metabolism by escalating reabsorption of calcium in the kidney, escalating mobilisation of calcium from the skeleton and escalating the production of 1,25$(OH)_2$D. This means that as a person becomes vitamin D insufficient and later deficient in the long term, the elevated levels of PTH result in normal to increased levels of 1,25$(OH)_2$D. This mechanism makes the measuring of 1,25$(OH)_2$D inappropriate as an indicator of vitamin D status.[2,6]

Long-term vitamin D status is thus not reflected by 1,25$(OH)_2$D levels. The measurement of 1,25$(OH)_2$D can however be used effectively in the diagnosis of certain acquired and inherited disorders in calcium metabolism as it relates to the alteration in the extra renal or renal production of 1,25$(OH)_2$D.[3]

Radioimmunoassay is the most widely used method to measure 25(OH)D.[6,44] Automated direct detection methods such as high-performance liquid chromatography, chemiluminescence and liquid chromatography–mass spectroscopy assay have become more popular, especially in large medical centres.[6] Automated, antibody- and microparticle-based chemiluminescent immunoassay methods using the LIAISON® 25 OH vitamin D assay and Abbott Architect assay have been clinically validated as accurate, rapid and precise tools in the measuring of 25(OH)D.[42,47] A number of authors point to the strengths of immunoassays running on automated platforms in terms of high output and convenience, especially for laboratories analysing large numbers of samples.[42,47] The measurement of 25(OH)D by immunoassay would remain the method of choice for reasons of turnaround speed, convenience and cost.[47]

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is seen as the gold standard of testing. The LC-MS/MS method has seen huge growth during the last 10–15 years. The method offers analytical specificity superior to that of the immunoassays or conventional high-performance/pressure liquid chromatography (HPLC), but sample throughput lags behind automated immunoassays.[48]

In a recent (2015) pilot study done by Vignali et al., work was done on the development of an algorithm to estimate individual vitamin D status, independently of serum 25(OH)D, using a simple questionnaire. The questionnaire mostly relied on indirect measurement of sunlight exposure. The algorithm performed extremely well with 212 of 235 (90.2%) of subjects assigned to the correct vitamin D status class. Four classes of vitamin D status were assumed: ≤10, 10–19.9, 20–29.9 and ≥30ng/mL. A larger study is planned with more varied population groups, different seasons, latitudes and urban and rural communities. This method might help select subjects that need serum 25(OH)D measurement.[49]

1.8 Functions of Vitamin D

1.8.1 Skeletal functions of vitamin D

The main and most recognised function of vitamin D is to maintain calcium and phosphorus homeostasis and to support bone mineralisation.[11,15,34,50] This is accomplished by increased calcium absorption in the intestinal tract, bone and kidney.[2,7,8,16] The small intestine is where dietary calcium will be absorbed and bone serves as a huge reservoir of calcium. Stimulating net resorption of bone minerals releases calcium and phosphate into the blood, and suppressing this effect allows calcium to be deposited in the bone. The kidney is vitally important in calcium homeostasis. Within normal blood calcium levels, almost all of the calcium that enters kidney glomerular filtrate is reabsorbed into blood, which will preserve blood calcium levels. If tubular reabsorption of calcium slows down, calcium will be by excreted into the urine.[16]

In a vitamin D-deficient state, the intestinal absorption of dietary calcium will be approximately 10–15% and in a vitamin D-efficient state, absorption will be approximately 30–40%. With increased demand, during periods of growth, pregnancy and lactation, as much as 60–80% can be absorbed.[2,7,9,16,18] On the other hand, if there is not enough

dietary calcium, $1,25(OH)_2D$ will interact with the vitamin D receptor (VDR) in the osteoblast in the bone which will ultimately lead to the mobilising of calcium stores from the skeleton to sustain serum calcium levels within the normal range.[7,8] The production of $1,25(OH)_2D$ is stimulated by parathyroid hormone (PTH). There is a negative response through calcium which reduces PTH and a direct negative response from $1,25(OH)_2D$ to PTH. The functioning metabolite $1,25(OH)_2D$ also shows swift actions through a membrane receptor.[19] $1,25(OH)_2D$ can aggressively enhance dietary phosphorus absorption. When phosphate and calcium are in a supersaturated state, it will result in mineralisation of the collagen matrix laid down by osteoblast. Although $1,25(OH)_2D$ does not have a direct active role in mineralisation, it is responsible for maintaining blood levels of phosphorus and calcium within the normal range so that mineralisation can take place.[7]

In adults low levels of 25(OH)D and high PTH lead to low calcium and phosphorus product, with consequential osteomalacia (derived from Greek, *osteo*, which means “bone” and *malacia* which means “softness”) which is a defective mineralisation of the collagen matrix, bringing about a reduction of structural support; it is also associated with an increased risk of fracture.[18] With the decline in 25(OH)D levels leading to secondary hyperparathyroidism associated with osteoclastogenesis (the development of osteoclast) and a subsequent increase in bone resorption that exceeds osteoblast-mediated bone formation, this can hasten and aggravate osteopenia and osteoporosis in adults. Vitamin D supports bone health by upholding the PTH levels at a physiologically healthy level, stimulating osteoblastic action and bone mineralisation, plus reducing risks of falls and in that way reducing the risk of fracture.[18] This effect seems to be mediated by improved muscle function and strength.[9,51]

Eight studies were included in the meta-analysis by Bischoff-Ferrari et al. on fall prevention and vitamin D supplementation. They concluded that vitamin D‘s effects are not limited to bone health alone. “Stronger bones are less likely to fracture”, as vitamin D supplementation (700 – 1000 IU/day) reduces the risk of falling amongst older individuals by 19%.[52] In addition to the role that vitamin D plays in promoting healthy bones and bone growth, it has also been established to reduce the risk of stress fractures in young female military recruits as this group may be especially vulnerable to stress fractures owing to their intense physical activity. Stress fractures can be reduced by 20% by supplementing with calcium (2000mg) and vitamin D (800 IU).[53]

The incidence of osteoporosis among the elderly is between 33–66%, depending on their age.[16] It is estimated that the lifetime risk of an osteoporotic fracture is 50% for women and 20% for men 50 years or older in their remaining lifetime.[26] Studies report a reduction of up to 52% of hip fractures with calcium (1200mg) and vitamin D (800 IU) supplementation.[9,16,35] It is documented that 52% of woman taking osteoporotic medications have vitamin D insufficiency (<30ng/mL). All osteoporotic patients taking osteoporosis medications need sufficient vitamin D and calcium to take full advantage of the benefits of these drugs.[9]

1.8.1.1 *Vitamin D and fracture healing*

Fracture healing can be divided into four stages that overlap: the inflammation stage (week 1), the soft callus-formation stage (week 2–3), the hard callus-formation stage (week 4–16), and the bone-remodeling stage (week 16 and beyond).[36] Each of these stages is characterised by specific complex cellular and molecular processes. Vitamin D has a role to play in every stage of fracture healing.[36]

Amongst older adults, it is recorded that about 30% fall each year and of these falls about 10% result in a fracture. These falls are a significant factor in morbidity and mortality in the elderly population.[11] According to the National Osteoporosis Foundation of America, about 24% of individuals aged 50 years and older with hip fractures die in the year after the fracture and about 20% of those individuals that were mobile preceding the hip fracture will now need long-term care. There was a significant reduction in hip fractures in those participants who received vitamin D and calcium supplementation (8 trials with >46 000 participants; RR=0.84; 95% CI: 0.73–0.96).[11] Vitamin D deficiency (36%) and insufficiency (78%) have been identified in fragility fracture patients. Lower vitamin D levels are associated with an increased risk of falling, diminished muscle strength,[54] low bone density, hip fractures and increased urine secretion of CrossLaps, a bone resorption marker.[55] Human bone turnover is a balanced process of bone formation and resorption. Most of the organic matrix of bone consists of Type I collagen. This is a helical protein that is cross-linked at the C- and N-terminal ends of the molecule. A fragment of collagen at the C-terminal end changes its nature; this fragment is called Beta-CTx (Beta–Crosslaps). During bone resorption proteases degrade collagen and this fragment is released in circulation. In bone resorption this marker is increased.[55]

These results confirm the importance of vitamin D screening and vitamin D supplementation guidelines for hospital fracture care pathways. The pathway recommends that orthopaedic surgeons prescribe 50 000 IU vitamin D on admission and on discharge the following: calcium carbonate (600mg), vitamin D (400 IU), and a multivitamin containing 400 IU vitamin D daily.[54]

Doetsch et al. demonstrated in a double-blind prospective study that vitamin D_3 (800 IU) and calcium (1000mg) supplementation (12 weeks) versus a placebo can have a positive effect (p=0.006) on the healing of a proximal humerus fracture (PHF) (top of the upper arm bone) as measured with bone mineral density (BMD) scanning.[56]

Numerous studies have shown that supplementation of calcium (500mg–1200mg/day) and vitamin D (400 IU–800 IU/day) reduces the risk of hip fracture by 26–58% and non-vertebral fracture incidence by 23 –32%.[9,52] There have been questions on the efficacy of calcium and vitamin D to reduce fracture risk; however on closer investigation it was found that subjects in the study were less than 60% compliant in taking their supplementation versus the Women's Health Initiative (WHI) (1000mg calcium and 400 IU Vitamin D) study where it was found that women who took their supplementation a minimum of 80% of the time had a 29% reduction in hip fractures. It was also noted that 400 IU is a far smaller dose (only 40%) than what is now recommended to maintain 25(OH)D levels of >30ng/mL.[9]

Approximately 5–10% of patients will have a certain amount of trouble with the final union of their fractures of which the most problematical is nonunion ("normal biological healing process ceases to the extent that solid bony union will not occur without further treatment").[58] Vitamin D deficiency has been shown to be linked with impaired fracture healing. Intestinal calcium absorption that is sub-optimal and increases bone resorption can occur with vitamin D deficiency; this deprives the fracture site of the necessary calcium it needs for mineralisation. This can play a part in the development of nonunion.[58] The diagnosis and treatment of vitamin D deficiency in nonunion of fractures is important owing to the role it plays in bone metabolism.[58,59]

A recent systematic review of the literature by Gorter et al. provides evidence that vitamin D affects the course of fracture healing, but the precise cellular role remains uncertain.They found a quantifiable positive effect in the form of improved bone mineral

density and improved fracture callus area at the fracture site. It is recommended that future research ought to focus on the clinical effects of vitamin D deficiency and the role of vitamin D supplementation on fracture healing.[36] Eschle and Aeschlimann also ask the question if vitamin D supplementation can benefit fracture healing and not only prevent fractures. The results of the specific literature search on vitamin D supplementation and fracture healing are limited in comparison with research on fracture prevention. The conclusion was that the benefits of vitamin D in fracture healing await additional trials, but all fractures in the elderly point towards the need for secondary prevention guidelines pertaining to falls, osteoporosis and vitamin D.[51] This then also begs the question if fractures and surgery will influence existing vitamin D status.

1.8.1.2 *Musculoskeletal function*

Vitamin D deficiency is linked to osteomalacia, in adults presenting with nonspecific complaints of melancholy, fatigue, body aches and bone pain, and this can be incapacitating to the patient. Symptoms of osteomalacia can be reversed by restoring vitamin D status.[2,6,16,17] Up to 40–60% of patients with fibromyalgia may have some element of osteomalacia and vitamin D deficiency.[2,16,17]

Vitamin D deficiency is linked to dispersed muscle pain, muscle weakness primarily in the proximal muscle groups (muscles closer to the body's midline), and a decrease in performance speed.[60] This originates from muscle wasting of mostly type II muscle fibres. Secondary hyperparathyroidism and resultant hypophosphatemia in severe vitamin D deficiency could also cause proximal muscle weakness. There is a positive association between 25(OH)D levels, proximal muscle strength, lower extremity function and physical performance. Postural and dynamic balance and muscle strength were increased by vitamin D supplementation.[18]

Several observational studies have reported an association between vitamin D insufficiency and poor lower extremity muscle performance, gait imbalance and increased risk of falls.[6,11,14,16,34,35] The current evidence does not conclude that there is a connection between vitamin D and chronic pain. The connection between vitamin D and chronic pain would offer a simple solution for the management of chronic pain, but the RCTs are still too few and supporting studies are inadequate.[61]

1.8.2 **Nonskeletal functions of vitamin D**

The disclosure in 1979 that most body tissues and cells contained vitamin D receptor unlocked an exciting new chapter regarding the potential biological functions of vitamin D. The vitamin D receptor (VDR) is present in the osteoblast, intestinal tract, lymphocytes, ß-islet cells and many other organs in the body, including the skin, brain, heart and prostate.[2,17,35] The local manufacturing of $1,25(OH)_2D$ is considered to be imperative for keeping cell growth in check and probably preventing the cell from becoming independent and developing into a cancer cell. Activated B and T lymphocytes have VDRs, and $1,25(OH)_2D$ is a very efficient modulator of the immune function, therefore the link with autoimmune diseases.[2,16]

Optimum vitamin D status may be imperative in a number of non-skeletal functions. Vitamin D deficiency may be a factor in glucose intolerance, cardiovascular disease, compromised immunity,[2,6,15,16,34,50,62] and a range of autoimmune disorders including multiple sclerosis, type I diabetes, rheumatoid arthritis, inflammatory bowel disease and Behçet's disease; asthma and cancer; as well as certain psychiatric disorders such as depression, schizophrenia and dementia.[16,50]

The 20 000-person VITAL trial, recently initiated, could help to determine if high doses of Vitamin D (2000 IU/day) will reduce the risk of cardiovascular disease (CVD), cancer and osteoporosis. At the 17th Workshop on Vitamin D, Chicago, IL, 2014, it was reported that VITAL had now completed the recruitment of over 26 000 participants who will be followed until 2017. A large portion of these participants will provide blood samples for 25(OH)D analyses and there are also 18 supplementary studies with this cohort. It is expected that over the next five years, VITAL, together with other large randomised trials of vitamin D supplementation worldwide, will provide essential data on the capability of vitamin D supplementation to influence disease mitigation in populations.[63] Another high dose (4000 IU/day) vitamin D trial in pre-diabetics is presently in the planning stages. Thus the position of vitamin D supplementation in nonskeletal disease must still be fully established.[63]

The recent systematic review by Bjelakociv et al. suggests that vitamin D (400 IU – 2000 IU daily) may reduce mortality by about 6%, which corresponds to approximately 200 individuals needed to be treated for two years for one additional life to be saved. Vitamin D also decreases cancer mortality in four per 1000 persons treated for 5–7 years.[64]

The evidence for the potential nonskeletal functions and benefits of Vitamin D is not as convincing as that for the skeletal effects, and conclusive evidence of these benefits awaits the outcome of more controlled clinical trials.[65]

1.8.2.1 *Cancer*

When comparing communities living at higher latitudes (with lower UV exposure and thus subsequent lower vitamin D status) with communities living at lower latitudes (with higher UV exposure and thus subsequent higher vitamin D status), living at higher latitudes is associated with a higher risk of the incidence of a variety of cancers.[18] Figure 1.4[66] is a graphic representation of all cancer death rates per 100 000. Countries closer to the equator seem to have a lower cancer death rate.[66] In a 2012 review of ecological studies associating solar UVB exposure / vitamin D and cancers, a strong inverse correlation was found with solar UVB and 15 types of cancer: Hodgkin's and non-Hodgkin's lymphoma, breast, cervical, ovarian, endometrial, vulvar, oesophageal, bladder, gastric, colon, rectal, lung, pancreatic and renal cancer.[18] These studies on association do have particular restrictions regarding the establishment of a causality as low vitamin D levels are also linked with other confounding aspects that are related to higher cancer risk, including lack of physical activity (correlated with less outdoor time and less UV solar exposure) and obesity (vitamin D is sequestered in adipose tissue).[18] A population-based, double-blind, randomised placebo-controlled trial with a duration of four years with 1179 postmenopausal women (>55 years old), where the principal secondary outcome was cancer incidence, showed that with supplementation of calcium (1400–1500mg/day) and vitamin D_3 (1100 IU/day) the reduction in relative risk (RR) of cancer was approximately 60% (p<0.01). With the repetition of a cancer-free survival analysis after the first 12 months it was revealed that the relative risk for the calcium + vitamin D supplementation group was reduced by approximately 77% (confidence interval (CI): 0.09–0.60; p<0.005).[67]

A PubMed database search done by Garland et al. yielded 63 observational studies of vitamin D status in relation to cancer risk, including 30 of colon, 26 of prostate, 13 of breast, and 7 of ovarian cancer, and several that assessed the association of vitamin D and cancer risk. In the majority of studies a protective relationship was found between sufficient vitamin D status and lower risk of cancer. The evidence suggests that improving

vitamin D status (for example by vitamin D supplementation), could decrease cancer prevalence and mortality at low cost, with no or few harmful effects.[68] Increasing evidence proposes a biological plausibility for anti-carcinogenic effects of vitamin D, which could explain these findings.[18] Although studies show promising results, there is still not conclusive evidence that vitamin D prevents the formation of cancer cells, slows down the progression of cancer, or helps prevent metastatic cancers.[69]

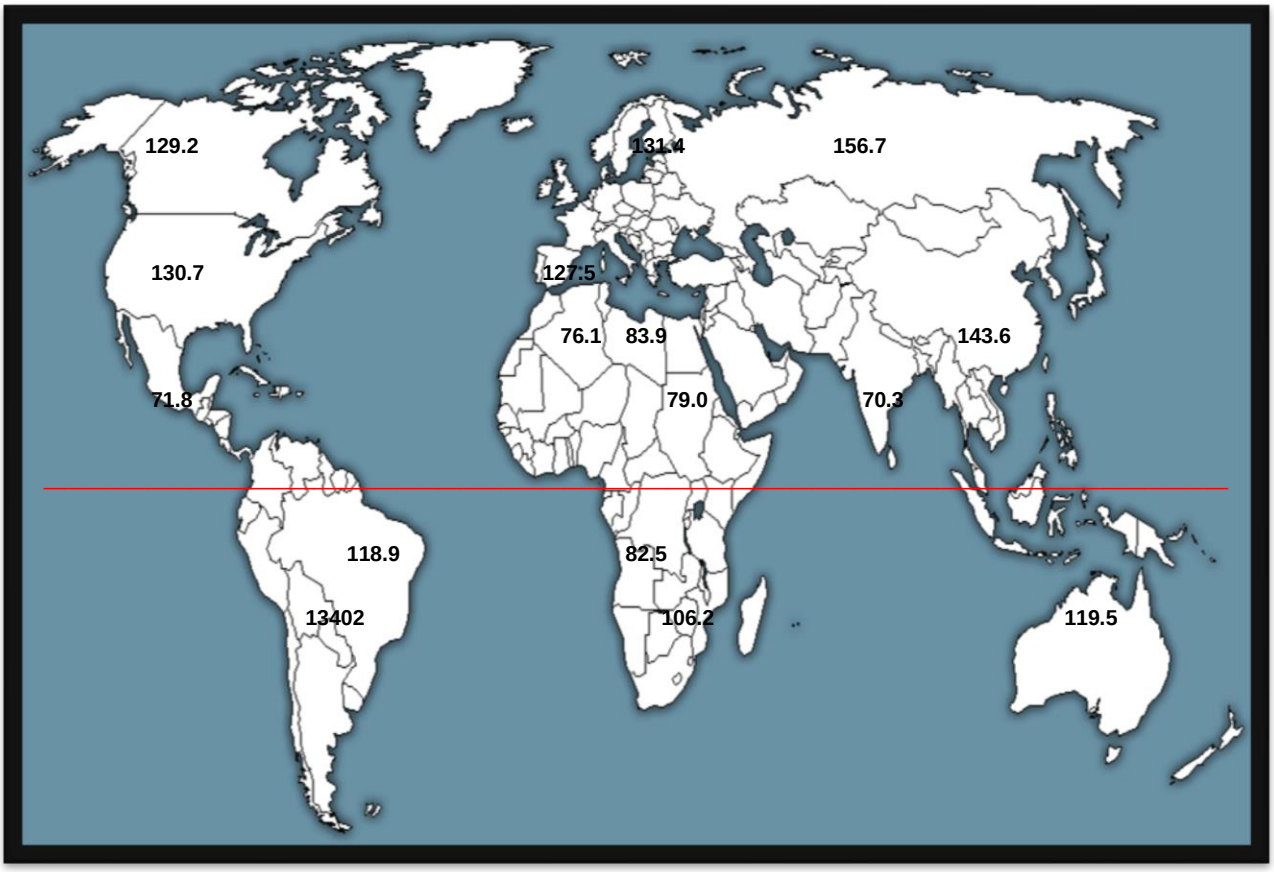


Figure 1.4: All Cancer Death Rate per 100 000 (2014)[66]

1.8.2.2 *Cardiovascular risk*

The role of vitamin D in cardiovascular health is a very new and unique field of interest.[70] There are many studies (prospective, epidemiological and meta-analytical) that suggest a significant inverse association between vitamin D serum levels and cardiovascular risk.[67,68] The prospective Intermountain Heart Collaborative Study (>40 000 participants) showed that levels of 25(OH)D of <15ng/mL when compared with levels of 25(OH)D of >30ng/mL

were associated with a significant rise in hyperlipidemia, hypertension, peripheral vascular disease, coronary artery disease, myocardial infarction, stroke, heart failure and type II diabetes as well as incident death.[18] This data is strongly supported by epidemiological and observational data that link vitamin D deficiency to the evidence, degree and prevalence of cardiovascular disease conditions and different risk factors. Very little is known about vitamin D as a preventative and/or therapeutic agent of cardiovascular disease. The limited data on vitamin D as therapy militate against any recommendations to support vitamin D supplementation as a therapeutic measure to improve cardiovascular outcomes.[69-71]

1.8.2.3 *Autoimmune disease*

In ecological studies it has been shown that the presence of certain autoimmune diseases (type I diabetes, multiple sclerosis (MS) and Crohn's disease) is associated with latitude. In these findings rests the potential role of sunlight and thus vitamin D status. At higher latitudes there is an increased prevalence for these autoimmune diseases (also inflammatory bowel disease and rheumatoid arthritis).[18] In one case control study it is shown that the risk of multiple sclerosis significantly decreased with increased levels of 25(OH)D (odds ratio for a 8ng/mL increase in 25(OH)D was 0.59 (95% CI: 0.36–0.97).[72] In another study addressing the effect of vitamin D on MS the safety of high-dose vitamin D (~14 000 IU/day) is highlighted. The noticeable immunomodulatory effects included a constant decrease in T-cell proliferation and the result was a trend towards a smaller number of relapse episodes.[68] There have also been studies where a greater intake of vitamin D was inversely associated with the risk of rheumatoid arthritis (RR 0.67; 95% CI: 0.44–1.00;p=0.05).[73]

In a nested cohort study an inverse association has been shown between maternal vitamin D levels and the risk of type I diabetes in the offspring. In this cohort of 30 000 pregnant women, the odds of type I diabetes in the women with the lowest quartile were more than twofold higher.[74] A birth-cohort study of children (>10 000) showed that regular supplementation (2000 IU vitamin D/day) in their first year of life was associated with a 88% reduction of the risk of type I diabetes later in life compared with children without vitamin D supplementation.[75] These links point towards the contributing role that vitamin D plays in the pathophysiology of certain autoimmune diseases. Immune cells have both the

enzymatic equipment to produce 1,25$(OH)_2$D and a VDR. This might explain why certain polymorphisms in the VDR gene appear to influence the risk of numerous autoimmune diseases, the time of commencement of the disease, and disease action.[18]

1.8.2.4 *Infectious disease*

Vitamin D has a plethora of effects when it comes to regulating the immune system and this plays a role in fighting infectious diseases. Vitamin D enhances the natural immunity against a variety of infections. Historically, cod liver oil (natural source of high amounts of vitamin D (500 IU/5ml)[12,38] was given to tuberculosis patients in the 19th and 20th century. Patients were also treated with heliotherapy (sun exposure) later in the 19th century. Niels Ryberg Finsen was awarded the Nobel Prize for medicine in 1903 for his contribution to the treatment of disease, particularly lupus vulgaris (tuberculosis of the skin) with concentrated light therapy. After the identification of vitamin D as the active ingredient in cod liver oil, it was used in several studies in the treatment of lupus vulgaris. In the antibiotic era, the use of heliotherapy and vitamin D treatment in tuberculosis patients was relegated to history.[18]

A reduction in cold and influenza symptoms was revealed in association with vitamin D supplementation in a study where the objective actually was to measure bone loss. These effects might be explained by the ability of 1,25$(OH)_2$D to enhance the natural immune system.[18]

1.8.2.5 *Respiratory diseases*

25(OH)D levels seem to correlate positively with lung function and asthma control and inversely with corticosteroid use. Very few intervention studies on vitamin D's effect on asthma exist, but one of them showed as a secondary result that vitamin D_3 (1200 IU/day) supplementation in children was linked to a significant reduced risk of asthma exacerbation. Vitamin D's immunomodulatory and pulmonary effects most probably play a role here.[18]

1.8.2.6 *Mortality*

In the narrative review of Pilz et al. the vast majority of prospective observational studies show that low 25-hydroxyvitamin D (25(OH)D) concentrations are linked with an increased risk of mortality. In the largest meta-analysis including more than 800 000 participants, the RR (95% CI) for mortality in the bottom versus the top third of baseline 25(OH)D levels was 1.35 (1.22–1.49). When comparing participants with 25(OH)D levels of <10ng/mL versus participants with ≥30ng/mL, the RR (95% CI) was 1.5 (1.21–1.87). When assuming a linear relationship between 25(OH)D and mortality, every decrease of 10ug/mL was associated with a 16% increase in all-cause mortality.[76]

1.9 Risk Factors for Vitamin D Insufficiency and Deficiency

There are numerous risk factors for hypovitaminosis D (Table 1.3). About 80% of the variation amongst individuals' vitamin D status can be attributed to skin synthesis as a function of UVB exposure and skin colour. With darker skin there is less cutaneous vitamin D synthesis.[6]

Table 1.3: Risk Factors for Vitamin D Insufficiency and Deficiency

Risk factor[2,6,13,16-18,24,34,50,85]	**Example**
Reduced skin synthesis	
Skin pigmentation	Dark skin
Limited UVB exposure	Latitude >37º north or south
	Season, time of day, weather
	Protective clothing, sunscreen
	Physical inactivity, homebound
	Air pollution
Decreased intake	Unhealthy diet
	Limited supplement intake
	Breastfeeding – low vitamin D content of human milk
	Intestinal malabsorption syndromes
	Parenteral nutrition
Increased catabolism	Glucocorticoids, antiepileptic drugs, HAART (AIDS treatment), antirejection medication
Obesity	Increased BMI decreases vitamin D status
	Sequestration of vitamin D in fat stores
Aging	Limited UVB exposure
	Decreased vitamin D intake
	Decreased cutaneous synthesis with age
Decreased synthesis of 25(OH)D	Liver failure
Reduced synthesis of $1,25(OH)_2D$	Chronic kidney disease
Heritable disorders	Pseudovitamin D deficiency rickets
	Vitamin D resistant rickets
Acquired disorders	Tumour-induced osteomalacia
	Primary hyperparathyroidism
	Hyperthyroidism

UVB = Ultraviolet B

BMI = Body Mass Index

HAART = Highly Active Antiretroviral Therapy

AIDS = Acquired Immune Deficiency Syndrome

25(OH)D = 25-hydroxyvitamin D

$1,25(OH)_2D$ = 1,25-dihydroxyvitamin D

The Fitzpatrick Skin Phototype Classification system is often used for skin classification.[2,87-91] The skin classification (Table 1.4) is done according to colour of skin and its ability to burn or tan. Skin classification is done as one of six skin phototype options.[87]

Table 1.4: Skin Classification

The Fitzpatrick Skin Phototype Classification[79]
Type FZ I: White skin, always burns, never tans
Type FZ II: White skin, always burns, minimal tan
Type FZ III: White skin, burns minimally, tans moderately and gradually
Type FZ IV: Light brown skin, burns minimally, tans well
Type FZ V: Brown skin, rarely burns, tans deeply
Type FZ VI: Dark brown/black skin, never burns, tans deeply

MED is the Minimal Erythemal Dose and is defined as the smallest dose of UVB that may produce pink erythema (sunburn) 24 hours after exposure. Supposing that unprotected skin has been exposed to UV radiation for the first time, to determine the MED, the response of the skin will be logged 24 hours after UV exposure. The smallest dose that brings on any visible reddening (pink) at that point is defined as one MED. Redness that happens immediately after UV exposure, however, and vanishes during the following 3–5 hours, is mainly the result of heat and is not equivalent to real UV erythema. On this basis the reading is not taken until 24 hours later.[92]

As an example: a young adult with skin type III who was exposed to 1 MED of 54mJ/cm^2 showed a 50-fold rise in blood concentrations of 25(OH)D within 8 hours of exposure, whereas an adult of the exact same age group with a skin type V that was exposed to the same amount of sun did not exhibit any considerable increase in circulating concentrations of 25(OH)D. The adult with skin type V will require 5–10-fold more sun exposure and only exhibit a 30-fold increase in blood concentrations of 25(OH)D to ~30ng/mL.[2] In general darker skin (greater melanin pigmentation) and skin that does not burn easily produce less Vitamin D.[8]

Latitude, time of day and season can significantly influence cutaneous vitamin D production. During the months of November to February there are striking decreases (~ 80–100% depending on latitude) in the number of UVB photons that reach the earth's surface – that is, above latitude North 37^o. Therefore very little or no vitamin D is manufactured in the skin during winter months. On the other hand, below 37^{o}N and closer to the equator, more vitamin D can be manufactured throughout the year, but in early morning and late afternoon the zenith angle of the sun's rays can be so slanted that very

little vitamin D is produced, even in summer.[2] South Africa's latitude is between 20º and 35º S.[65,93] Johannesburg's coordinates are 26.2044 ºS, 28.0456ºE and Cape Town's coordinates are 33.9253 ºS, 18.4239ºE.[94]

Sunscreen lotions work by absorbing UVB and some UVA radiation ahead of its entering the skin. A sunscreen with an SPF (Sun Protection Factor) of 8 can decrease the skin's capacity to produce vitamin D by >95%; sunscreens with an SPF of 15 can decrease the skin's capacity by up to 98%.[2]

Vitamin D_3 is a fat-soluble vitamin and therefore stored in body fat. Excess vitamin D_3 that is produced during sun exposure can be stored in body fat to be used during winter months when skin production is reduced. However, for obese individuals, the fat can be an irretrievable sink for vitamin D as vitamin D is sequestrated in fat tissue, increasing their risk of vitamin insufficiency and deficiency.[2] It has been shown that with vitamin D supplementation of 50 000 IU, obese subjects show improved blood levels of vitamin D of no more than 50%, compared with those of non-obese individuals.[2]

Low vitamin D intake, advanced age, disease and medication can affect vitamin D metabolism.[3,8] Gastrointestinal diseases such as Crohn's disease and Coeliac disease can influence vitamin D absorption. Vitamin D status is negatively influenced by pancreatic insufficiency with its associated malabsorptions, for example, cystic fibrosis and cholestatic liver disease such as primary biliary cirrhosis. Chronic kidney disease is commonly associated with low vitamin D status, to some extent owing to reduced action of 25(OH)D-1∝-hydroxylase. Nephrotic syndrome might cause increased urinary losses of vitamin D and vitamin D binding protein.[3]

It is therefore expected that vitamin D deficiency would be more common in high-risk populations[6] such as persons who are older, sun deprived, and have sub-optimal nutrition, overweight and obese individuals, and those with dark skin such as black and Hispanic people.[3,8]

According to Pramyothin and Holick[95] the following candidates are at risk of vitamin D deficiency and screening can be warranted in patients with the following conditions:[81]

(1)Rickets

(2) Osteomalacia

(3) Osteoporosis

(4) Chronic kidney disease

(5) Hepatic failure

(6) Malabsorption syndromes:

- (A) Cystic fibrosis
- (B) Inflammatory bowel disease
- (C) Crohn's disease
- (D) Bariatric surgery
- (E) Radiation enteritis

(7) Hyperparathyroidism

(8) Medications:

- (A) Antiseizure medications
- (B) Glucocorticoids
- (C) AIDS medications
- (D) Antifungals, e.g., ketoconazole
- (E) Cholestyramine

(9) African-American and Hispanic children and adults

(10) Pregnant and lactating women

(11) Older adults with history of falls

(12) Older adults with history of non-traumatic fractures

(13) Obese children and adults (BMI > 30 kg/m^2)

(14) Granuloma-forming disorders:

- (A) Sarcoidosis
- (B) Tuberculosis
- (C) Histoplasmosis *(List adapted from[81])*

1.10 Vitamin D Requirements

The 2011 report of the Institute of Medicine (IOM) on dietary reference intakes for calcium and vitamin D set the adequate intake of vitamin D at 400–800 IU/day (10–20µg/day), based on age.[18,37,79] The Scientific Advisory Committee (SACN) on Nutrition 2016 report on Vitamin D and health recommends a Reference Nutrient Intake (RNI) of 400 IU/day for

the UK population aged 4 years and above. According to the SACN, this is the average amount of vitamin D needed by 97.5% of the population to maintain serum 25(OH)D levels above 10ng/mL (25nmol/L) when UVB sunshine exposure is minimal.[85] Dietary Reference Intakes (DRI) compiled by the Nutrition Information Centre at Stellenbosch University are set at much lower levels of 200–600 IU/day based on age.[96] However there is growing consensus that vitamin D intakes above these levels are associated with better health outcomes.[35,84] The Endocrine Society sets a much higher daily requirement of 1500–2000 IU/day (Table 1.5).[18,37] Universal experience with vitamin D demonstrates that vitamin D supplementation of 600 IU/day (as the body's sole input of vitamin D) would not be enough to produce even 10 ng/mL, let alone 20 ng/mL or above. To achieve a level of >30ng/mL 25(OH)D, an intake of at least 800–1000 IU of vitamin D is needed, depending on age.[84] The response increment of vitamin D is 1–0.7ng/mL/100 IU.[18,84] The tolerable upper intake level (UL) for daily vitamin D according to the DRI is 2000 IU and the UL set by the IOM is 4000 IU[18,37,81] (Table 1.5). The Endocrine Society sets tolerable upper intake levels at 10 000 IU.[37]

Veugelers and Ekwaru recently published an open letter in *Nutrients* (2014) titled: "A Statistical Error in the Estimation of the Recommended Dietary Allowance for Vitamin D".[94] In their calculations they found that the regression line revealed that 600 IU of vitamin D/day achieve that 97.5% of individuals will have serum 25(OH)D values above 10.7ng/mL (26.8 nmol/L) rather than >20ng/mL (50nmol/L) as currently assumed. It is then estimated that 8895 IU of vitamin D per day may be needed to accomplish 97.5% of individuals to achieve serum 25(OH)D values >20ng/mL(50 nmol/L). As this dose is far greater than the range of studied doses, caution is warranted when interpreting this new estimate. Nevertheless, the very high estimate only illustrates that the dose is well in excess of the current RDA of 600 IU/day and even the tolerable upper intake of 4000 IU/day.[18,37] It is stated that with the current recommendation of 600 IU/day of the IOM, bone health objectives and disease and injury prevention targets will not be met. This is demonstrated in two studies conducted in Canada with total vitamin D intake of 632 IU/day (dietary intake 232 IU/day and supplementation of 400 IU/day), where 10% of participants had values of <20ng/mL (<50nmol/L). The second study reported serum 25(OH)D levels of <20ng/mL (<50nmol/L) for 15% of participants who reported supplementation with vitamin D. If the IOM's RDA had been adequate, these percentages should not have been greater than 2.5%. These studies then show that the current public health targets are not being

met.[94] The authors recommend "that the RDA for vitamin D be reconsidered to allow for appropriate public health and clinical decision-making".[94]

Table 1.5: Vitamin D Recommendations for Adults 18 years to >70 years

Gender	Age	DRI AI[96]	DRI UL[96]	DRI NOAEL[96]	DRI LOAEL[96]	IOM EAR[18,74, 96]	IOM RDA[18,37,81]	IOM UL[18,37]	Endocrine Society[18,37,81] Daily Requirement	NRV[97] (4 years and older)
	Years	IU/day	IU/day	IU/day	IU/day	IU/day	IU/day	IU/day	IU/day	IU/day
Male	18–30	200	2000	2400	3800	400	600	4000	1500–2000	
	31–50	200	2000	2400	3800	400	600	4000	1500–2000	
	51–70	400	2000	2400	3800	400	600	4000	1500–2000	
	>70	600	2000	-	-	400	800	4000	1500–2000	
Female	18–30	200	2000	2400	3800	400	600	4000	1500–2000	600
	31–50	200	2000	2400	3800	400	600	4000	1500–2000	
	51–70	400	2000	2400	3800	400	600	4000	1500–2000	
	>70	600	2000	-	-	400	800	4000	1500–2000	

1µg *=40 IU*
DRI *=Dietary Reference Intake*
AI *= Adequate Intake*
UL *= Tolerable Upper Intake Level*
NOAEL *= No Observed Adverse Effect Level*
LOAEL *= Lowest Observed Adverse Effect Level*
IOM *= Institute of Medicine*
EAR *= Estimated Average Requirements*
RDA *= Recommended Dietary Allowance*
NRV *= Nutrient Reference Values*

1.11 Vitamin D and Sun Exposure

The exposure of the skin to sunlight will provide most humans with their vitamin D needs. Ageing diminishes the skin's ability to produce vitamin D_3, because the vitamin D forerunner, 7-dehydrocholesterol, decreases with age. The ability of the human skin to manufacture vitamin D_3 is so sufficient that even a 70% drop in the skin's 7-dehydrocholesterol levels by age 70 does not stop the elderly from producing adequate quantities of vitamin D_3 in their skin from casual sun exposure outdoors.[2,14] It should be

noted that a sunscreen with a sun protection factor (SPF) of 30 can absorb about 99% of the UVB radiation responsible for making vitamin D_3.[14]

A single session of sunlight exposure to the whole body (in a bathing suit), just sufficient to produce erythema (minimal redness to the skin) (1 minimal erythemal dose), yields about 10 000–25 000 IU vitamin D_3.[7,35,86] Increased skin pigmentation can reduce the skin's production of vitamin D[37,98] as shown in the study done between South Asian and white groups by Farrar et al., where the recommended exposure to sunlight did not produce sufficient vitamin D status in the Asian group.[98] Melanin absorbs both visible and UV radiation as revealed by in-vitro studies where UVB transmission in black epidermis is less than a quarter than that in white epidermis.[99]

There can be large fluctuations in serum 25(OH)D levels from late summer to late winter. A drop of 0.11ng/mL per day was recorded by Barger-Lux and Heaney, a fall from 48.8 to 29.6ng/mL over 175 days.[86] In Cape Town, South Africa, the study of Haarburger et al. showed no significant difference between the medians (18.8ng/mL versus 19.5ng/mL) for summer versus winter values.[30]

The highest 25(OH)D attainable by sun exposure is in the region of 60– 80ng/mL.[15,35] Similar results were found by Barger-Lux and Heaney.[86] Among Nebraskan outdoor labourers the three highest measurements reported were between 81 and 84ng/mL. The assay used in this study measured other vitamin D metabolites as well as 25(OH)D and consequently resulted in higher values.[15]

Current clinical recommendations indicate the following: Exposure of arms and legs, not face (without sunblock) to sunlight, 15–30min two or three times a week between the hours of 11:00 and 15:00 during the spring, summer and autumn for individuals with skin type II or III, (depending on time of day, season, latitude and skin pigmentation).[2,16,31,35,] For example, at latitudes above 37ºN, from about mid-October to mid-March, the solar angle is such that no vitamin D is converted in the skin.[35] For these reasons high amounts of sun exposure will not always ensure acceptable levels of vitamin D adequacy.[15,86] This implies that the common clinical recommendation for sun exposure may not ensure vitamin D sufficiency in all population groups.[15]

The Endocrine Society's Clinical Practice Guidelines also recommend sensible sun exposure, which in many cases is the most important source of vitamin D for most individuals, provide a list of vitamin D-rich food, and encourage taking a daily supplement of vitamin D to ensure adequate 25(OH)D levels.[18,37]

The Scientific Advisory Committee on Nutrition states in its 2016 report that "it is not possible to make any recommendations regarding the amount of sunlight exposure that would be required during the summer to maintain serum 25(OH)D concentration ≥10ug/mL(25nmol/L) in 97.5% of the UK population during the following winter because of the number and complexity of factors that affect endogenous vitamin D production".[85]

1.12 Vitamin D Supplementation

Oral vitamin D supplements are obtainable as vitamin D_3 or D_2. Vitamin D_3 supplementation is usually favoured over D_2 because D_2 may be less bioactive and may have lesser affinity for the VDR, but data are inconsistent.[6] In the systematic review and meta-analysis of Tripkovic et al., it was indicated that supplementation with vitamin D_3 had a significant and positive effect in the raising of 25(OH)D serum levels when compared with the effect of vitamin D_2 (mean difference: 15.3nmol/L; 95% CI: 6.12,24,34; p=0.001).[100] The only high-dose vitamin D available in South Africa is prescription formulation of vitamin D_2, Lennon-Strong calciferol tablets (50 000 IU), and calciferol oily solution – Lennon (5 000 IU/ml), both Schedule 3.[6] The guidelines with regard to the quality, safety, dosage and efficacy requirements for the registration of health supplements are published by the Medicines Control Council (MCC) of South Africa. The guidelines have recently been updated. The permissible levels and claims for vitamins are contained in the MCC Complementary Medicines – Health Supplements Quality, Safety and Efficacy document Version 2 published in June 2016. The first version was published in November 2014 and opened for comments until 26 February 2015.[101] The document is available at http://www.mccza.com/Publications/Index/1.The maximum vitamin D dose that is allowed in an over-the-counter supplementation is 1000 IU.[101] Metagenics® D_3 5000 IU and 2000 IU are dispensed on prescription, but are not scheduled. Table 1.6 gives a list of vitamin D supplements available in South Africa. Many multivitamins also contain vitamin D, but not in therapeutic doses.[39]

The evidence for proposed vitamin D supplementation to maintain sufficient 25(OH)D serum levels (>30ng/mL) in the absence of adequate sun exposure remains inconclusive. Previous recommendations were 1000 IU/day of vitamin D_3,[8,16] but a more recent six-month supplementation trial suggests that up to 4000 IU/day may be needed to produce serum levels >30ng/mL.[8]

The Endocrine Society suggests that all adults aged 19–70 years and 70 years or older require at least 600 and 800 IU/day respectively, of vitamin D. Whether 600 and 800 IU/day vitamin D will be enough to provide all the potential non-skeletal health benefits is not known at this time. However, to elevate the blood level of 25(OH)D above 30ng/mL may demand at least 1500–2000 IU/day supplemental vitamin D per day.[37]

Table 1.6: Supplemental Sources of Vitamin D in South Africa

Supplement Source	Amount (per tablet)	
	µg	IU
Cholecalciferol (D_3)		
Multivitamin (various preparations available)	2.5–10	100–400
Certain calcium supplements (package insert)	10–12.5	400–500
D-Vit® Tabs (Georen) (package insert)	10	400
D-Drops® (Georen) (/0.5ml) (package insert)	10	400
Vitamin D_3 (SOLAL®) (package insert) (2014)	12.5	500
Vitamin D_3 (SOLAL®) (package insert) (since 2016)	25	1 000
Vitamin D (ReVite™)	12.5	500
Vitamin D_3 (Biogen®) (package insert)	12.5	500
Vitamin D complex (Foodstate®) (Package insert)	12.5	500
Vitamin D_3 drops (Colief®) (/drop) (package insert)	2	80
D_3 5000 (Metagenics/Amipro) (package insert)[a]	125	5000
D_3 2000 Complex (Metagenics/Amipro) (packageinsert)[a]	50	2000
D_3 1000 (Metagenics/Amipro) (package insert)	25	1000
D_3 liquid (Metagenics/Amipro) (/drop) (package insert)	25	1000
Calciferol (D_2)		
Lennon-Strong calciferol tablets (Schedule 3) (package insert)	1250	50 000
Calciferol oily solution – Lennon (Schedule 3) (package insert)	125	5 000
Injectable preparations		
Cernivit® multivitamin preparation for intravenous or intramuscular injection (5ml vial) (D_3) (package insert)	5.5	220
Intramuscular vitamin D is not available in South Africa as a standard formulation[39]		
Other preparations		
One-Alpha® capsules 1µg[b] (alphacalcidol) Adcock Ingram (Schedule 4) (package insert)	1	
One-Alpha® capsules 0.25µg[b] (alphacalcidol) Adcock Ingram (Schedule 4) (package insert)	0.25	
Rocaltrol® 0.25µg and 0.5µg capsules (calcitriol) Roche (Schedule 4) (vitamin D metabolite)	0.25 0.5	
Vitamin D_3 transdermal patch[c] (NeoGenesis Health) (1patch / day) (package insert)	125	5000
Vitamin D_3 cream[40] (not available in South Africa)		

[a] *Metagenics vitamin D_3 only available on prescription but not scheduled*

[b] *Active form of vitamin D, mostly used in kidney disease*[43]

[c] *Current clinical trial underway to test if a transdermal patch will safely and successfully deliver vitamin D_3 in humans. ClinicalTrials.gov Identifier: NCT02174718.*[41]

The Endocrine Society Clinical Practice Guidelines suggest one 50 000 IU capsule of vitamin D_2 per week for 8 weeks,[8,18] followed by 50 000 IU every 2–4 weeks thereafter, is a cost-effective approach to correct vitamin D deficiency and maintain sufficient levels.[8] Otherwise ~6000 IU/day of vitamin D_3 or D_2 should be taken to achieve blood levels above 30ng/mL. This must be followed by maintenance therapy of 1500–2000 IU/day.[37] In obese patients, malabsorption syndromes and patients on medication that can affect vitamin D metabolism, 2–3 times higher dosages (6000–10 000 IU/day) of vitamin D are suggested to treat vitamin D deficiency. This must be followed with maintenance therapy of at least 3000–6000 IU/day(Table 1.7).[37,81] The approach of supplementing with 50 000 IU of vitamin D fortnightly to prevent recurrence and treat vitamin D insufficiency and deficiency was followed for six years without toxicity.[18]

Table 1.7: Recommended treatment strategies according to the Endocrine Society Clinical Practice Guidelines for adults with vitamin D deficiency

Age / Co-morbidity	**Recommendation: Using vitamin D_2 or D_3**[37,81]
Adults over 18 years	50 000 IU[a] once a week for 8 weeks, followed by 50 000 IU every 2–4 weeks thereafter or 6000 IU daily for 8 weeks to achieve a blood level of vitamin D >30ng/ml followed by maintenance therapy of 1500–2000 IU/day.
Obesity, malabsorption, or medications affecting vitamin D metabolism	Two to three times higher dose; at least 6000–10 000 IU per day to achieve a blood level of vitamin D >30ng/ml followed by maintenance therapy of 3000–6000 IU/day.
Primary hyperparathyroidism	Treatment as needed, with serial monitoring of serum calcium. Most patients will not increase their serum calcium, and serum PTH[b] may even decrease.

[a]*IU International unit*

[b]*PTH:Parathyroid hormone*

In the narrative review of Pilz et al., observational data and meta-analysis of RCTs found that vitamin D_3 supplementation reduces overall mortality. Supplementation of any type of vitamin D versus placebo reduced mortality, but vitamin D_3 supplementation was associated with significant mortality reduction and a risk ratio (95% CI) of 0.94 (0.91–0.98). Based on this finding, it was calculated that 150 individuals needed to be treated with vitamin D_3 over five years to prevent one death. These findings provide a strong rationale in favour of the safety and benefits of vitamin D supplementation.[76]

1.13 Vitamin D Intoxication

Vitamin D intoxication cannot be caused by excessive exposure to sunlight because any excess previtamin D_3 or vitamin D_3 is destroyed by sunlight.[8,16,101]

Toxicity from vitamin D supplementation is uncommon and consists mainly of acute hypercalcaemia and hyperphosphataemia, with doses greater than 10 000–50 000 IU per day; related serum levels of 25(OH)D are >150–200ng/mL.[2,3,8,16,35] Two cases of intoxication were recently reported by Araki et al., where the cause of the intoxication was due to labelling and manufacturing errors of two supplements made in the United States. Even with toxic dose estimated at 100 000 IU/day for a month, these two cases were taking nine and 18 times more respectively.[103] Clinical symptoms include lethargy and confusion, muscle weakness, nausea, vomiting and dehydration.[35,104] Vitamin D intoxication is associated with the hyper-absorption of phosphorus and calcium. Manifestations of vitamin D toxicity include ectopic soft tissue calcification, including vascular calcification, hypercalcaemia, hypercalciuria, nephrocalcinosis, and renal failure.[95]

No cases of vitamin D toxicity have been reported with vitamin D supplementation doses less than 10 000 IU/day (approximately 120–200 IU/kg body weight/day).[35] The risk of vitamin D intoxication is and stays extremely rare in healthy adults, unless intoxication is accidental.[95]

1.14 Sun Exposure and Cancer Risk

According to the World Health Organization (WHO) Global Burden of Disease from Solar Ultraviolet Radiation (UVR) Report, globally excessive UVR exposure contributes to 0.1% of the global burden of disease [1.5 million Disability-Adjusted Life Years (DALYs)] where the greatest burden results from UVR-induced cortical cataracts, cutaneous malignant melanoma, and sunburn. It should be noted that the counterfactual of zero UVR exposure will not result in a lesser disease burden, but will rather result in a higher disease burden owing to diseases from vitamin D deficiency.[105]

There are three major types of skin cancer: Basal Cell Carcinoma (BCC), consisting of raised, translucent pearly nodules that occur mostly on the face and other exposed areas;

Squamous Cell Carcinoma (SCC), usually with raised, pink opaque patches or nodules that can frequently form sores or ulcers in the centre and appear most often on exposed areas of the body; and Cutaneous Melanoma (CM), comprising small brown, black or multi-coloured patches that have an irregular outline. These can occur from pre-existing skin spots or moles or can appear in normal skin.[106]

Australia has the highest incidence of skin cancer in the world, followed by South Africa's white population, with about 20 000 reported cases every year and about 700 deaths. Reports by the WHO estimate that there are between 2–3 million non-melanomas and about 132 000 cutaneous melanomas globally every year.[106]

In South Africa the highest annual incidence of all three types of skin cancer occurs in the white population, followed by coloured, Asian and black population groups. BCC and SCC are twice as common in males as in females, and melanoma is the least frequent skin tumour, with BCC the most frequent diagnosed skin tumour.[107]

The relationship between sun exposure and skin cancer (BCC and SCC) has been established since 1927, and since 1955 for melanoma.[108] The relationship between the different types of skin cancer and sun exposure is quite complex as there are many factors to consider. There is the relationship with ambient solar irradiance, which includes population location, personal residence history, and migration. Then there is also the relationship with cutaneous sun sensitivity, which includes ethnic origin, colour of unexposed skin, and the ability to tan. There is also the relationship with personal sun exposure and body areas exposed to the sun. This includes total lifetime exposure, recent total exposure, occupational exposure, non-occupational exposure, and sunburn.[108]

The Cancer Association of South Africa (CANSA) contends at least 80% of sun-induced damage to the skin occurs before the age of 18 and manifests later in life. Therefore skin protection is very important in children. People with light skin, red hair and skin spots or moles, as well as people with a family history of skin cancer, are considered to be at high risk.[106] Tanning booths and sun beds can double the melanoma risk of an individual and can thus not be considered a safe alternative to sun exposure.[106] CANSA advocates the avoidance of direct sunlight between 10:00 and 15:00, the use of sunscreen and protective clothing, and the avoidance of sunlamps and tanning parlours.[106]

The relationship between skin cancer and sun exposure remains very complex. Juzeniene et al. found that occupational sun exposure does not increase the risk of CM, but that CM rates are more related to intermittent and intense sun exposure and sunburn than the total dose of sun exposure. Sun exposure actually leads to melanin generation and skin thickening, both of which are presumably protective factors. Sun exposure also generates vitamin D that is probably anti-melanogenic. It is evident in incidence-exposure curves that zero exposure does not result in a zero incidence. The fact that there is similarity in density between melanomas on sun-exposed areas and sun-shielded areas only emphasises the need for more research in the area to identify non-UV factors that are associated with skin cancer. Current analyses do not identify “safe” UV doses where the risk of skin cancer is not present. The avoidance of sun exposure has the possibility to decrease the incidence of skin cancer in fair-skinned populations.[109]

1.15 Conclusion

A renowned vitamin D researcher, Dr MF Holick, states that “vitamin D deficiency is now recognized as one of the world’s most common medical conditions”.[14] In many countries throughout the world mean serum vitamin D levels are around 20ng/mL; this implies that vitamin D insufficiency exists in about 50% of these populations.[24] The 25-hydroxyvitamin D (25(OH)D) assay is the most-ordered hormone assay in the United States.[14,35]

Current research has revealed that optimal vitamin D status may be essential for a range of skeletal and non-skeletal outcomes.[2,6] Vitamin D can no longer be just the nutrient for the prevention of rickets among children, but vitamin D should be regarded as necessary for overall health and wellbeing.[2]

The present body of evidence is significant enough to validate that health care professionals should be encouraged to recognise the vitamin D deficiency pandemic and to take appropriate steps to prevent and treat this widespread condition[14] by recommending sensible sun exposure, food containing vitamin D in the diet, and vitamin D supplementation.[18] Increasing and maintaining a sufficient 25(OH)D (>30ng/mL) worldwide in the general adult population without rare conditions associated with an increased risk of vitamin D toxicity will help to improve overall health and wellbeing and should be a main concern.[2,18] A ‘healthy lifestyle’ that includes outdoor activities with carefully balanced

sunlight exposure, together with efforts to combat the global burden of obesity, would substantially improve vitamin D levels in the general population, without dietary vitamin D interventions.[76]

1.16 Rationale

The current body of research on vitamin D and its skeletal and non-skeletal functions sparked a new interest in this long-forgotten vitamin. Data on vitamin D status in South African adults is limited[114] and on examining data from other sunny countries[15,50] it can be concluded that we cannot just assume that the population will be vitamin D sufficient.

In 2011 Parry et al. conducted vitamin D status screening in pre-operative orthopaedic paediatric patients and ascertained that 90% of patients had insufficient vitamin D levels, while 51% were deficient.[44]

To the author's knowledge, in South Africa vitamin D status in the pre-operative adult orthopaedic private patient has not been investigated. Also, obese patients are operated on an average of ten years earlier than their normal BMI counterparts. The vitamin D status of orthopaedic patients can influence their orthopaedic outcome in the future as vitamin D has been linked to falls and fractures. Identifying patients or populations as at risk groups can help prevent future major surgical interventions. South Africa has abundant sunshine, but there are many other factors that can influence vitamin D status, such as sun exposure (clothing and use of sunscreen), skin pigmentation, and dietary vitamin D intake, to name but a few. This study aims to shed some light on these factors by assessing vitamin D status pre- and also post-operatively, thus examining the effect of the recovery period after the surgical intervention on vitamin D status.

In choosing orthopaedic surgical patients as the population for this study, a number of questions may be addressed, namely, the:

- determine if the orthopaedic surgical population is an ‘at risk’ population
- vitamin D status of this population;
- vitamin D oral intake of this population;
- sun-exposure habits of this population; and
- impact of minor and major orthopaedic surgical interventions and the recovery period on vitamin D status.

CHAPTER 2: METHODS

2 Methods

2.1 Aim and Objectives

2.1.1 Aim

To determine the vitamin D status of orthopaedic surgical patients and the impact of the surgical intervention on vitamin D status after the recovery period.

2.1.2 Objectives

2.1.2.1 To determine the vitamin D status of orthopaedic patients on admission to a private hospital in Johannesburg, South Africa.

2.1.2.2 To determine if there is a relationship between 25(OH)D and the following:

- Demographic parameters (gender, age, smoking and skin classification)
- Anthropometric status (BMI and waist circumference)
- Sun exposure (calculated sun index)
- Vitamin D intake (supplementation and dietary intake)

2.1.2.3 To determine vitamin D status after the surgical intervention.

2.1.2.4 To determine if there is a change in the following:

- Sun exposure (calculated sun index) after surgical intervention (comparison of sun exposure [calculated sun index] before and after surgery)
- Vitamin D intake after surgical intervention (comparison of vitamin D intake before and after surgery)

2.2 Hypotheses

2.2.1 The orthopaedic surgical population have insufficient vitamin D levels and can be classified as an ‘at risk’ group.

2.2.2 There is no statistically significant relationship between 25(OH)D pre-operatively as well as post-operatively and:

- Demographic parameters (gender, age, smoking, and skin classification)
- Anthropometric status (BMI and waist circumference)
- Sun exposure (calculated sun index)

- Vitamin D intake (supplementation and dietary intake).

2.2.3 There is no statistically significant change in sun exposure (calculated sun index) after orthopaedic surgical intervention compared with the pre-surgery status.

2.2.4 There is no statistically significant change in vitamin D intake after orthopaedic surgical intervention compared with the pre-surgery intake.

2.2.5 There is no statistically significant change in 25(OH)D after orthopaedic surgical intervention compared with the pre-surgery status.

2.3 Study Plan

2.3.1 Study type

Descriptive observational cohort study with an analytical component.

2.3.2 Study population

Adult orthopaedic patients at Netcare Mulbarton Hospital in Johannesburg, Gauteng, South Africa.

2.3.3 Sampling method

Purposive sampling

2.3.4 Sample selection and size

The sampling frame consisted of adult orthopaedic surgical patients at Netcare Mulbarton Hospital, Johannesburg, Gauteng. The total required sample size was calculated in consultation with a statistician (Professor DG Nel) of Stellenbosch University. To determine statistically significant change in vitamin D status, sun exposure and vitamin D intake after orthopaedic surgical intervention compared with the pre-surgery status, a minimum of 67 patients was required according to the one-way ANOVA power analysis

with a small-to-medium effect size (RMSSE=0.40) with 90% power. The same group was used in pre- and post-operative testing.

2.3.5 **Inclusion criteria**

- All adult orthopaedic patients admitted to the hospital for elective orthopaedic surgical procedures
- Age: 18 – 75 years
- Consent given

2.3.6 **Exclusion criteria**

- Major orthopaedic trauma patients
- Immobile and/or bedridden patients
- Institutionalised patients
- Patients with the following medical conditions that could influence vitamin D status:[16]
    - Renal impairment
      Nephrotic syndrome
    - Chronic kidney disease stages 2–4
    - Liver failure
    - Liver dysfunction of 90% or more
    - Gastrointestinal surgery that influences absorption
- Patients using the following medication that could influence vitamin D status:[16]
    - Anticonvulsants
    - Glucocorticoids
    - Highly Active Antiretroviral Therapy (HAART) (HIV/AIDS treatment)
    - Anti-rejection medication
    - Steroids: long-term or high doses
- Rare conditions affecting vitamin D status:[16]
    - Inheritable disorders: e.g. resistant rickets and autosomal dominant hypophosphatemic rickets
    - Acquired disorders: e.g. tumour-induced osteomalacia

2.4 Methods of Data Collection

2.4.1 General procedure

Patients were identified from orthopaedic surgeons' theatre rosters with the help of the surgeons' assistants (Figure 2.1). Patients were invited to participate in the study in the ward after hospital admission. Once the patient had agreed to participate, arrangements were made to meet with them in the surgical ward before the scheduled surgery.

At the first meeting the study was explained in detail and written consent was obtained (Addendum A). All questionnaires were in English; however the investigator was able to translate any questions into Afrikaans during the interview.

Anthropometric and biochemical measurements were taken pre-operatively and all relevant questionnaires (anthropometric and biochemical, demographic, skin classification, sun exposure and short food questionnaire for vitamin D) were completed (Addenda B– F). Arrangements were made to have the blood sample taken pre-operatively. Lancet Laboratories (located on the hospital premises) were called and a Lancet sister took the blood sample in the surgical ward.

The second meeting was arranged telephonically where the patient was asked to visit Lancet Laboratories at Mulbarton Hospital for the post-operative blood sample. The follow-up visit was done 55–120 days later. The previously planned follow-up period of about a month did not coincide with follow-up visits with the orthopaedic surgeon, as most (88%) of the surgeries performed were minor surgeries that required no or few follow-up visits. The planned period of approximately a month was also extended as it was revealed in the literature that the whole-body, half-life of radio-labelled vitamin D is approximately two months.[45] The post-operative study population consisted of 23 participants (34% of pre-operative group). Follow-up response was quite low, despite numerous reminders, phone calls and messages. Patients were lost to follow-up because of their unavailability, not arriving for scheduled appointments, and transport difficulties. It is suspected that the research follow-up did not coincide with the surgical follow-up because of the large number of minor orthopaedic surgeries (88%). Surgical follow-up in these cases was often between one and two weeks. The proposed follow-up for the research was much later (7–17 weeks). This longer timeframe was planned to take into account the long half-life of

vitamin D (up to six weeks) and the timeframe was extended in an attempt to increase follow-up numbers.

At the second visit the investigator met the patients at Lancet Laboratories where the relevant questionnaires were completed (sun exposure and short food questionnaire for vitamin D) (Addenda E and F). If the investigator was unable to meet the patient at Lancet, the questionnaires were completed telephonically within 48 hours of the blood sample being taken.

All data collection components were undertaken by the researcher (dietician) with the help of a nursing sister (drawing of blood samples only).

2.4.2 Anthropometric measurements

Anthropometric measurements (Addendum B) were taken during the first meeting (pre-operative) in the surgical ward of Mulbarton Hospital at the patient's bedside with curtains drawn to ensure privacy. Measurements were taken by the researcher (dietician). See Table 2.1 for details of the measurements.

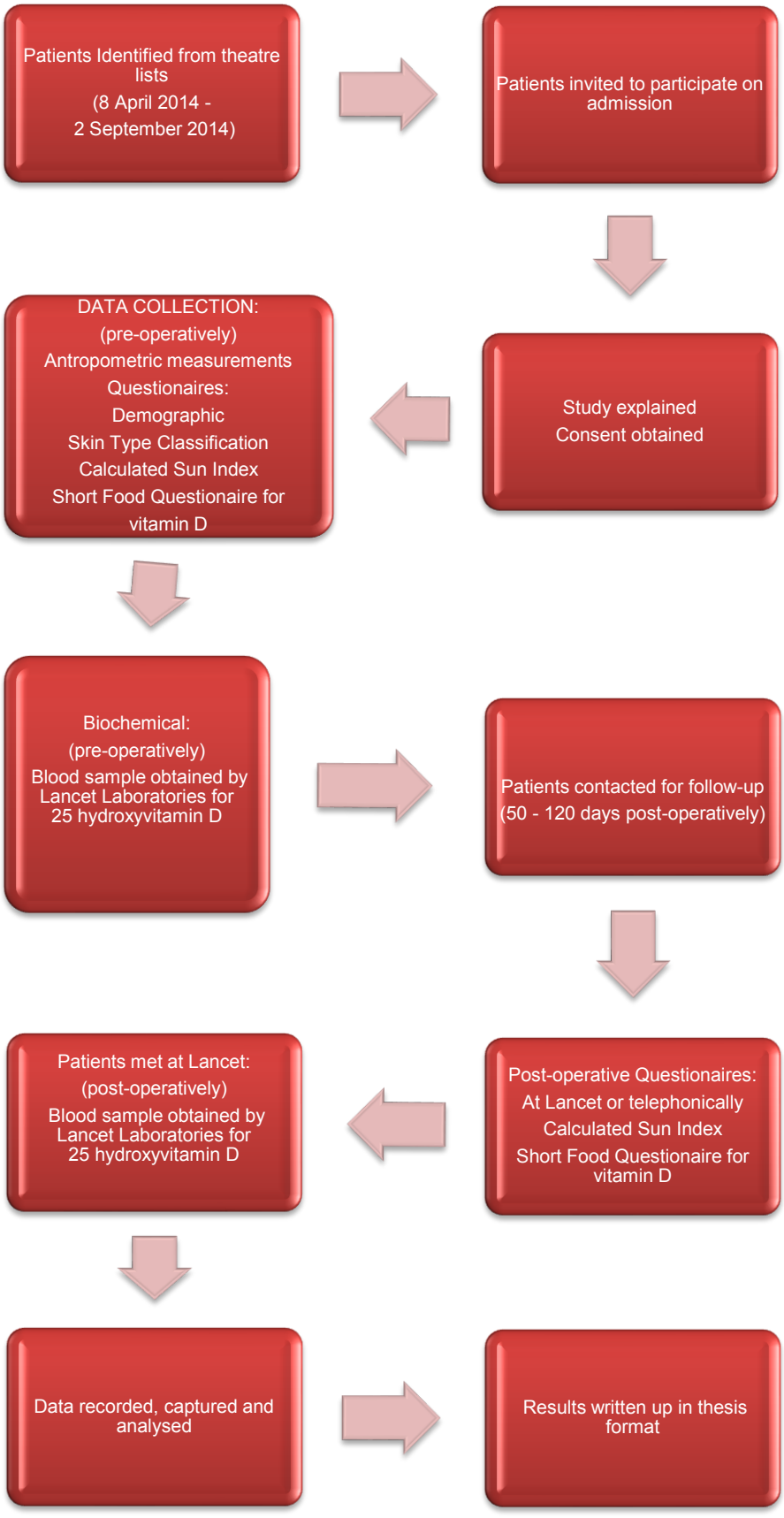


Figure 2.1: Conceptual Framework of Study Plan

Table 2.1: Anthropometric Measurements

Measurement & Equipment	Details of measurement[111]	Standardisation and reliability techniques
Weight Electronic scale: SECA Serial Number: 5703158071668	• Scale was placed on a flat hard surface. • Measurements done after bladder had been emptied and before a meal. • Measurements without shoes and with minimal clothing (theatre gown). • Subject stood in the centre of the platform and looked straight ahead, standing unassisted, relaxed but still. • Presence of notable oedema was recorded. • Body weight was recorded to the nearest 0.1kg. • Three measurements were taken (average used)	• Checked and adjusted to zero balance before each measurement.
Height Stadiometer: SECA Serial Number: 5703158071668	• Nothing coved the head. • Patient looked straight ahead with Frankfurt plane horizontal. • Shoulders were relaxed. • No shoes or socks. • Feet were together and flat on the floor. • Knees were straight. • Heels, buttocks and shoulder blades were in contact with the vertical surface of the stadiometer. • Arms were hanging loosely at the sides with palms facing the thighs. • Subjects were asked to take a deep breath and stand tall to aid the straightening of the spine. • Shoulders were relaxed. • The movable headboard was gently lowered until it touched the crown of the head. • The height measurement was taken at full inspiration. • Height was recorded to the nearest millimetre. • Three measurements were taken (average used).	• Standardised stadiometer used.
Waist circumference Tape measure	• Measurement was taken over minimal clothing. • Measurement was taken at the natural waist i.e. midway between the tenth rib (the lowest rib margin) and the iliac crest. • In obese subjects measurements were taken at the umbilical level, as it was difficult to gauge the waistline. • Subjects stood erect with abdomen relaxed, arms at the sides, feet together and weight equally divided over both legs. • Subjects were asked to breathe normally and to breathe out gently at the time of the measurement. • Measurements were taken without tape compressing the skin. • Readings were taken to the nearest millimetre. • Three measurements were taken (average used).	• Flexible non-stretch fibreglass tape.

2.4.3 **Questionnaires**

Four questionnaires were used in this study to obtain information: 1. Demographic Questionnaire (Addendum C); 2. Skin Type Classification (Addendum D); 3. Sun Exposure (Addendum D); 4. Short Food Questionnaire for Vitamin D (Addendum F).

All questionnaires were completed by the investigator (dietician) in a face-to-face interview in a private setting to ensure confidentiality on the day of admission to the surgical ward. All questionnaires were available in English; however the investigator was able to translate any questions into Afrikaans during the interview. The majority of patients at Netcare Mulbarton Hospital are English or Afrikaans literate, thus English and Afrikaans were adequate for communication. The questionnaires were compiled by the investigator and based on the latest literature. For the follow-up visit, patients visited Lancet Laboratories for the biochemical assessment. This opportunity was used to complete the sun exposure (Addendum D) and short food questionnaire for vitamin D (Addendum F) again. If the investigator was unable to conduct interviews at Lancet, the patient was telephoned within 48 hours to complete the questionnaires telephonically.

2.4.3.1 *Demographic questionnaire*

The demographic questionnaire (Addendum C) consisted of 19 questions. These included questions related to personal information (gender, age, date of birth, language and ethnic group) (six questions), medical history (chronic disease, medication and supplementation) (eight questions), smoking habits (four questions), and planned surgery (one question).This questionnaire was only completed once (pre-operatively).

2.4.3.2 *Skin-type classification*

Patient skin-type classification (Addendum C) was determined in the first interview on admission. The Fitzpatrick Skin Phototype Classification system is widely used for skin classification.[54,56] Skin classification (see Table 1.3) is done according to colour of skin and its ability to burn or tan. Skin classification is done as one of six skin phototype options (Figure 2.2)[112]. Questions included identifying colour of skin as white, light brown, brown or dark brown/black; and burning as always burns, burns minimally, rarely burns or never

burns. Final questions were on tanning, including never tans, minimal tan, moderate and gradual tan, tanning well, or deep tan. This questionnaire was only completed once (pre-operatively).

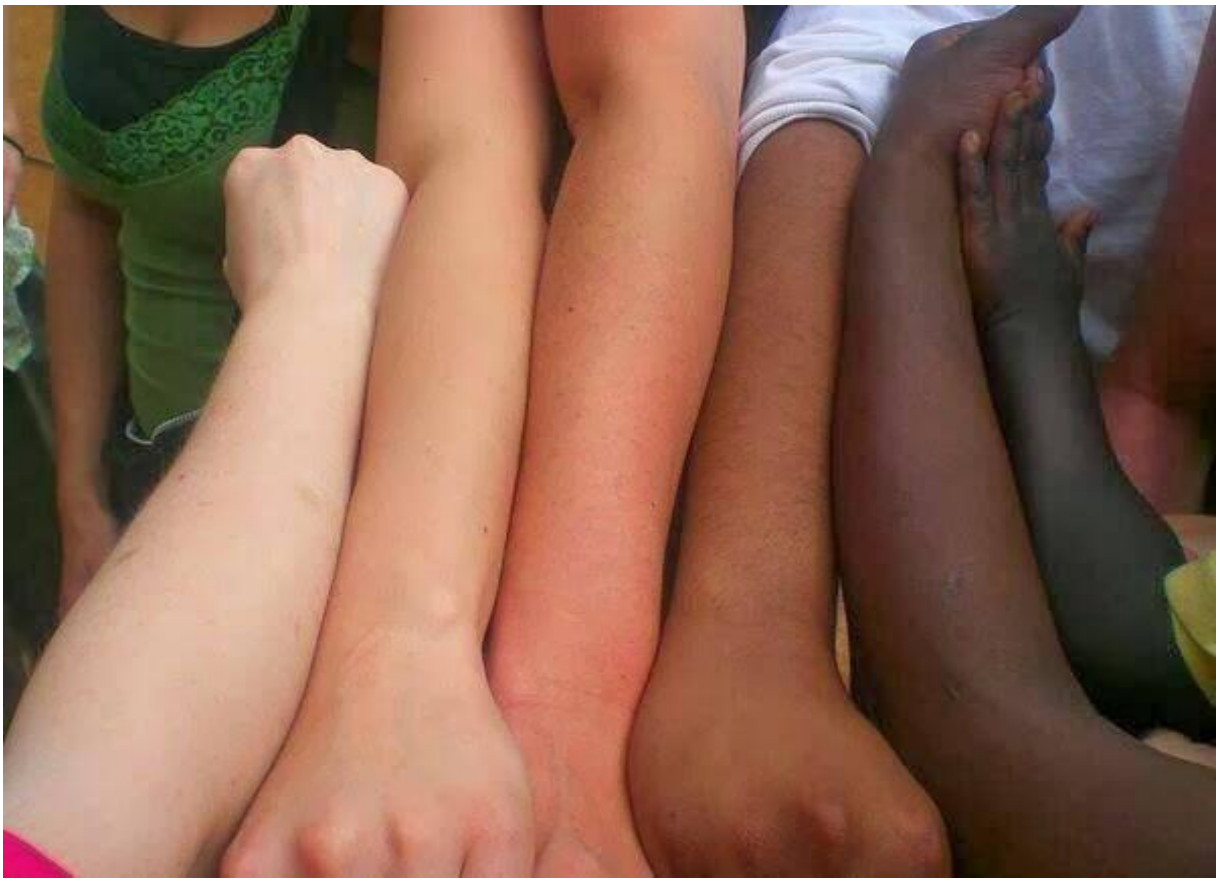


Figure 2.2: Graphic Illustration of the Fitzpatrick Skin Phototype Classification[112]

2.4.3.3 *Sun exposure*

Patient-calculated sun index (sun exposure) (Addendum E) was determined in the first interview pre-operatively and again at the follow-up in a direct interview or telephonically (post-operatively) to determine if habits had changed after the surgical intervention.

Sun exposure was calculated as follows: Calculated sun index = self-reported hours of sun exposure per week (Table 2.2) multiplied by the fraction of Body Surface Area (BSA) exposed to sunlight.[15,86,113] (Table 2.3).

Clinical recommendation: exposure, not face (without sunblock) to sunlight 15–30min two or three times a week (30min X 3 = 1.5 hours); exposure of arms (0.14 BSA) and legs (0.24 BSA) (Total BSA = 0.14 + 0.24 = 0.38).[2,16,31,35]

A calculated sun index of 0.615 is obtained with sun exposure equivalent to the clinical recommendation. (1.5 X 0.38 = 0.615). A calculated sun index below or above the clinical recommendation could then be compared to vitamin D status.

Table 2.2: Self Reported Sun Exposure per Week[114]

Time spent outdoors between sunrise and sunset	**With sunblock**	**Without sunblock**
Weekdays		
< 15 minutes / day		
15–30 minutes / day		
30–60 minutes / day		
1–2 hours / day		
>2 hours / day		
Weekends		
< 15 minutes / day		
15–30 minutes / day		
30–60 minutes / day		
1–2 hours / day		
>2 hours / day		
Calculated hours per week		

Sun exposure during the previous month on weekdays and weekends between sunset and sunrise, with or without sunblock.[114]

Table 2.3: Body Surface Area (BSA) Exposed to Sunlight[86]

Body Area	Adapted "rule of nines"[a]	Category 1			Category 2		Category 3	
		No shirt	Long-sleeved shirt	Short-sleeved shirt	Short pants	Long pants	No hat	Hat
Both arms	0.18	0.18	0.04	0.14				
Both legs	0.36				0.24	0.00		
Anterior trunk	0.18	0.09	0.00	0.00	0.00	0.00		
Posterior trunk	0.18	0.09	0.00	0.00	0.00	0.00		
Head	0.09						0.07	0.03
Perineum	0.01				0.00	0.00		
Column Totals	**1.00**	**0.36**	**0.04**	**0.14**	**0.24**	**0.00**	**0.07**	**0.03**

[a] The "rule of nines" estimates sectors of adults' BSA as percentages that are multiples of 9.[15,86] The rule was adapted to estimate the fraction of BSA exposed to sunlight by each subject's usual outdoor attire, where the attire consisted of one selection from each of the categories.[15,86,115,116]

2.4.3.4 *Short food questionnaire for Vitamin D*

The patients' dietary vitamin D intake (Addendum F) was determined in the first interview on admission and again on follow-up to determine if habits had changed after the surgical intervention.

The short food questionnaire for vitamin D was adapted from Blalock et al.[117] Permission to adapt and use the questionnaire was obtained from the author (24 January 2012). The basic format of the form was retained (how often and how much of a food item was eaten). The form was adapted by the addition of South African foods with the highest vitamin D content (13 changes were made). These foods were identified from the Condensed Food Composition Tables for South Africa[38] and FoodFinder 3 Software.[21] Vitamin D fortification is not very common in South Africa. Only margarine, health bars and certain cereals are fortified.

The purpose of this questionnaire was to determine the vitamin D intake from food over the past three months. First, for each food listed (19 foods), mark the column to show how

often, on average, the patient had eaten the food during the past three months. Secondly, mark whether the usual serving size was small, medium, or large. Specify margarine, health bar and cereal brands (Addendum F).

Average daily vitamin D intake was calculated from data collected and compared with DRI (Dietary Reference Intake) and Vitamin D status.

2.4.4 **Biochemical**

Blood collection was done by the trained staff of Lancet Laboratories according to their specified procedures (Addendum G).[118] Vitamin D status was determined with serum level of 25-hydroxyvitamin D by Lancet Laboratories using the ABBOTT ARCHITECT 25OH TOTAL VIT D Assay (an automated chemi-luminescent immunoassay).[119]

A follow-up vitamin D test was done after 55–120 days post-operatively at Lancet Laboratories, Mulbarton Hospital. Patients were contacted telephonically on numerous occasions to remind them of follow-up blood tests for vitamin D testing. After 120 days, patients that did not return for blood tests were recorded as lost to follow-up.

Lancet Laboratories use the following cut-off points on their pathology reports for vitamin D25: severe deficiency, <10ng/mL; moderate deficiency, 10–29ng/mL; sufficiency, 30–100ng/mL; and toxic, >100ng/mL.

2.5 Analysis of Data

2.5.1 **Statistical analysis of data**

Data was recorded on a pre-coded data-collection sheet, then captured in Microsoft Office Excel 2007 and processed to obtain results.

Data was analysed with the assistance of a statistician, Ms Tonya Esterhuizen from the Biostatistics Unit, Centre for Evidence-Based Health Care, Stellenbosch University. SPSS Version 22 (IBM® 2013) was used to analyse the data.

Summary statistics were used to describe the variables. Distribution of variables was presented with histograms and frequency tables. Medians and means were used as measure of central location for ordinal and continuous responses. Standard deviations and quartiles were used as indicators of spread. When data was skewed, median and interquartile ranges were used rather than mean and standard deviation.

A *p*-value of $p<0.05$ indicated statistical significance in hypothesis testing. Scatter plots were used to illustrate correlation before calculating the correlation coefficient. If the variables were normally distributed between two variables, the Pearson correlation coefficient was calculated (age and gender). If either or both of the variables had a skewed distribution or had outlying values, a non-parametric correlation coefficient was calculated based on the ranks of the observation (Spearman's rank correlation coefficient). Other tests used were *t*-test, one-way ANOVA, Wilcoxon signed-rank test, chi-square test and Fisher's exact test.

Correlations between vitamin D status and calculated sun index (sun exposure) and vitamin D intake were made. Correlations were made between pre- and post-operative vitamin D status, vitamin D dietary intake, and sun exposure habits.

2.5.2 **Demographic data analysis**

The demographic questionnaire was coded for data recording. Frequency tables and a scatter plot with linear regression were used to present data. Data was then summarised in tables. The correlation coefficient according to Pearson's correlation and the *t*-test was used to describe demographic data and its relation to vitamin D status.

2.5.3 **Anthropometric data analysis**

The anthropometric data was interpreted and classified according to the following variables (Table 2.4) and data capture sheet Addendum B was used. Mean, minimal and maximal values and standard deviation (SD) were used. Data was presented in a table and in bar charts. The Pearson correlation coefficient was used to determine correlation with vitamin D status.

Table 2.4: Anthropometric Measurements and Reference Values[111,120,121]

Anthropometrical Measurement	Interpretation and Classification			Calculation / Equation
Body Mass Index (BMI) (kg/m²) Calculated	**Classification**	**BMI**	**Disease Risk**	Formulae: BMI = weight / (height)² Weight in kilograms Height in metres
	Underweight	<18.50	Increased risk	
	Normal weight	18.50–24.99	Least risk	
	Overweight	25.00–29.99	Increased risk	
	Obese Class I Class II Class III (Extreme obesity)	 30.00–34.99 35.00–39.99 ≥40	 High risk Very high risk Extremely high risk	
Waist circumference (cm)	**Increased risk of metabolic complications[121]**		**Substantially increased risk of metabolic complications[121]**	N/A
	Females: ≥ 80cm		Females: ≥ 88cm	
	Males: ≥ 94cm		Males: ≥ 102cm	

2.5.4 Skin-type data analysis

Skin-type classification of the population was done according to the Fitzpatrick Skin Phototype Classification (Table 1.3). Frequency and percentage values were used to describe skin-type data. Data was presented in tables and bar charts. Mean values were used to describe vitamin D status according to skin type. One-way ANOVA tests were used to compare skin type to vitamin D levels. Line graphs were used to present data.

2.5.5 Sun exposure (calculated sun index) data analysis

Data was grouped and analysed according to calculated sun index. The median and interquartile ranges were used as data was skewed. Data was presented in tables, box plot graphs, scatter plot and receiver operating characteristic (ROC) curve. Spearman's

rank correlation coefficient was used between calculated sun index and vitamin D status to indicate correlation.

2.5.6 Vitamin D oral intake data analysis

Oral intake of vitamin D was grouped and analysed according to average daily intake and compared with vitamin D status. Mean, minimal and maximal levels were mentioned; however median and interquartile ranges were used as data was skewed. Oral intake was correlated with vitamin D status. Data was summarised and presented in tables and in a bar chart. Spearman's rank correlation coefficient was used between oral vitamin D intake and vitamin D status to indicate correlation.

2.5.7 Biochemical data analysis

Vitamin D status data was used, interpreted and classified according to variables presented in Table 2.5. Mean and standard deviation were used to describe data. A chi-square test was used to compare pre-operative values with post-operative values.Data was presented in tables, box plot figures, scatter plots, and a pie graph.

Table 2.5: Biochemical Measurements and Reference Values

Biochemical Measurement	**Interpretation and Classification**[2,3,6,8,9,24,34,37,86]
25-hydroxyvitamin D [25(OH)D]	Undetectable: <5ng/ml (12.5nmol/l)
	Vitamin D deficiency: <20ng/ml (<50nmol/l)
	Vitamin D insufficiency: 20–30ng/ml (50–75nmol/l)
	Sufficient vitamin D: >30ng/ml (>75nmol/l)
	Vitamin D toxicity: >100ng/ml (>250nmol/l)

2.6 Ethical and Legal Aspects

Ethics approval was obtained from the Health Research Ethics Committee of Stellenbosch University (S13/10/187). The protocol and ethics approval from Stellenbosch University were submitted to the Netcare Research Committee, a subcommittee of the Academic Advisory Board within Netcare, for approval. Approval was granted by Netcare (UNIV-

2014-0011).The hospital manager of Mulbarton Hospital granted permission for the study as part of the Netcare approval process.

2.6.1 **Informed consent**

Autonomy was upheld by means of a consent form (Addendum A). All participants completed a consent form where research aims and details of the study were explained. The consent form included permission to participate in the study, answer questionnaires, be measured and weighed, and for a blood sample to be taken for vitamin D analysis. Participation was stated as voluntary and all collected data was treated as private and confidential at all times. All patient data was stored in the private offices and on the private computer of the investigator. Only the investigator, research supervisors and statistician had access to the data.

Confidentiality and privacy were ensured at all times. All measurements and interviews were done in a private area. All data was handled confidentially. No names were attached to data, as all participants were allocated a research number.

CHAPTER 3: RESULTS

3 Results

3.1 Study Population

During the baseline phase of the study period (8 April–2 September 2014), 167 orthopaedic surgical patients at Mulbarton Hospital were on the theatre lists. Of these, 21 were excluded owing to age and language, and a further 79 were excluded owing to various reasons as indicated in Figure 3.1. This resulted in 67 patients included in the study.

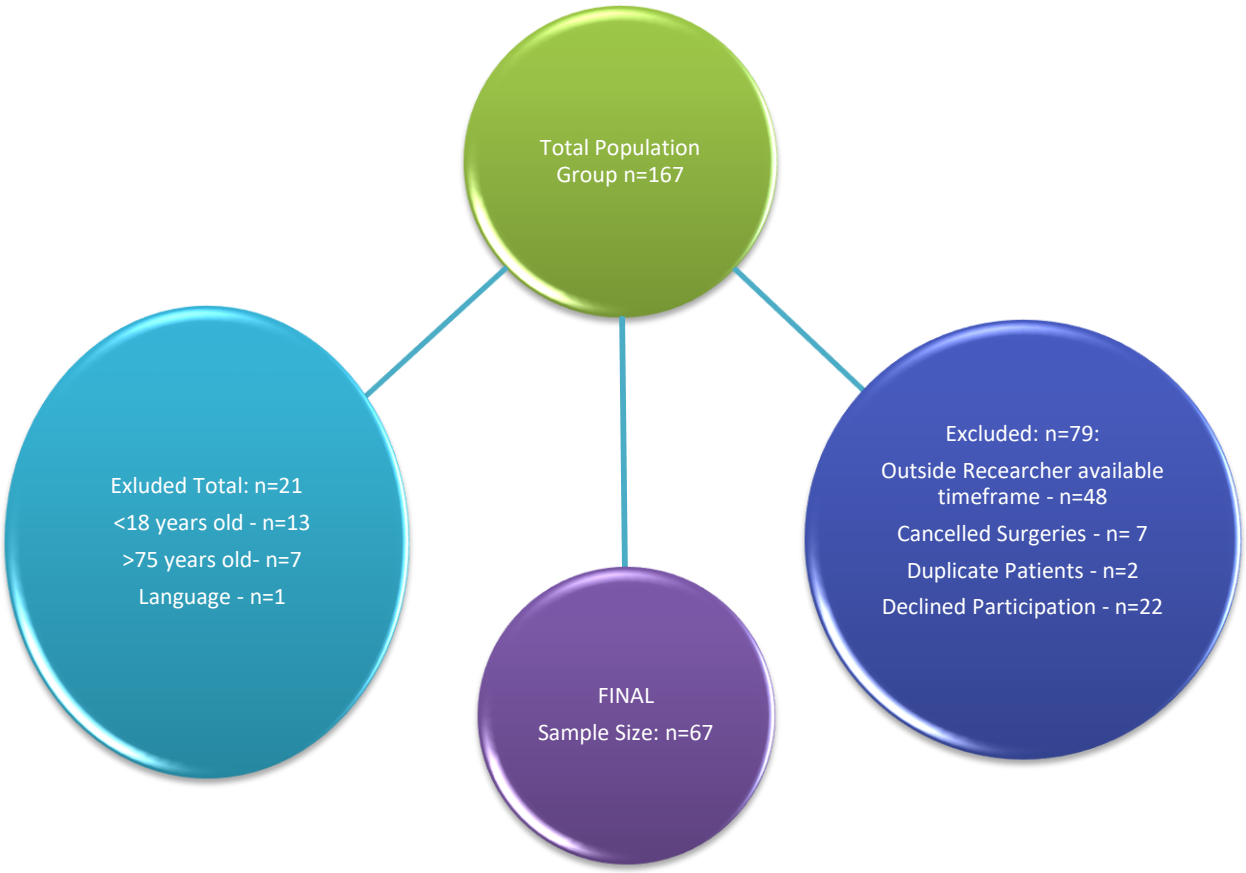


Figure 3.1: Patient Recruitment Flow Diagram

The mean timeframe for follow-up (round 2) was 78 days (SD 25; minimum 50 days and maximum 120 days) post-operative. Sixty-six percent of the study population was white, 15% black, 13% coloured and 6% Indian. Of these patients, 23 (34%) returned for follow-up data collection. Patients were lost to follow-up owing to lack of interest, unavailability, not appearing for follow-up appointments, appointments not correlating with doctors' appointments, and transport problems.

3.1.1 **Demographic data**

The demographics of the orthopaedic surgical patients included in the study are summarised in Table 3.1. Of the 67 patients, 32.8% were male and 67.2% were female. The mean age of the study participants was 50.94 (SD 12.32) years (range 20 to 74 years). Ninety-one percent were younger than 65 years and 9% older than or equal to 65 years of age. The vast majority of the patients could understand either English or Afrikaans as their first or second language. All patients were admitted for elective surgeries, with the bulk of surgeries being minor surgeries (88.1%) (Table 3.1).

Table 3.1: Demographic Characteristics of Patients

Patient Demographics	***n* (%)**	***n* (%)**
Gender	**Male** 22 (32.8%)	**Female** 45 (67.2%)
Age 50.94 (SD 12.32)	**< 65** 61 (91%)	**≥ 65** 6 (9%)
Language	**First Language** English 40 (59.7%) Afrikaans 18 (26.9%) isiZulu 4 (6%) Other 4 (6%) isiXhosa1 (1.5%)	**Second Language** English 26 (38.8%) Afrikaans 32 (47.8%) Other 6 (9%) Portuguese 2 (3%) isiXhosa1 (1.5%)
Type of elective surgery	**Minor Surgery 59 (88.1%)** Foot 13 (19.4%) Hand 13 (19.4%) Shoulder 13 (19.4%) Knee Scope 11 (16.4%) Elbow 4 (5.9%) Hip 3 (4.5%) Leg 1 (1.5%) Other 1 (1.5%)	**Major Surgery 8 (11.9%)** Knee 6 (9%) Hip 2 (3%)

SD = Standard Deviation

3.1.2 **Chronic disease profile of study population**

Self-reported chronic diseases (Table 3.2) of the study population were identified on the demographic questionnaire (Addendum C). Thirty-five participants (52.2%) of the study population identified one or more chronic conditions and 32 participants (47.8%) had no

chronic condition. The two most prevalent chronic conditions found were hypercholesterolemia (*n*=19, 28.4%) and hypertension (*n*=15, 22.4%).

Table 3.2: Chronic Conditions of Study Population

Chronic Condition	***n***	**%**
Hypercholesterolemia	19	28.4
Hypertension	15	22.4
Thyroid	12	17.9
Diabetes Mellitus	7	10.4
Arthritis	5	7.5
Receiving hormonal replacement therapy	4	6
Asthma	4	6
Ulcer	3	4.5
Gout	3	4.5
Diverticular Disease	1	1.5

3.1.3 **Smoking habits of study population**

The majority of the study population did not smoke at the time of data collection (*n*=51, 76.1%),while 9 (13.4%) had previously smoked. Of the current smokers (*n*=16, 23.9%), 6 (37.5) smoked 10 or fewer cigarettes per day. Most of the smokers (*n*=13, 81%) had been smoking for more than 10 years (Table 3.3).

Table 3.3: Smoking habits of the Study Population

Smoking Habit	***n* (%)**
Smoker	16 (23.9%)
Non-smoker	51 (76.1%)
Previous smoker	9 (13.4%)
Smoking > 10 years	13 (81%)
Smoking < 10 years	3 (9%)
Smoking > 10 cigarettes/day	10 (62.5%)
Smoking ≤ 10 cigarettes/day	6 (37.5%)

3.1.4 Anthropometric data of the study population

The mean weight of the males (*n*=22) was 94kg (SD 19) with a minimum of 65.8kg and maximum 148.3kg. For the females (*n*=45) the mean weight was 84kg (SD 26) with a minimum and maximum weight of 48.7kg and 151.6kg respectively (Table 3.4).

Table 3.4: Anthropometric Data of the Study Population

	Gender							
	Male (*n*=22)				**Female (*n*=45)**			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Height (m)	1.80	0.10	1.61	1.86	1.60	0.10	1.49	1.75
Weight (kg)	94.0	19.0	65.8	148.3	84.0	26.0	48.7	151.6
Waist (cm)	103.3	14.6	84	145	94.9	16.8	69	132
BMI (kg/m²)	30.5	6.1	20.8	47.3	31.7	8.9	18.0	51.8

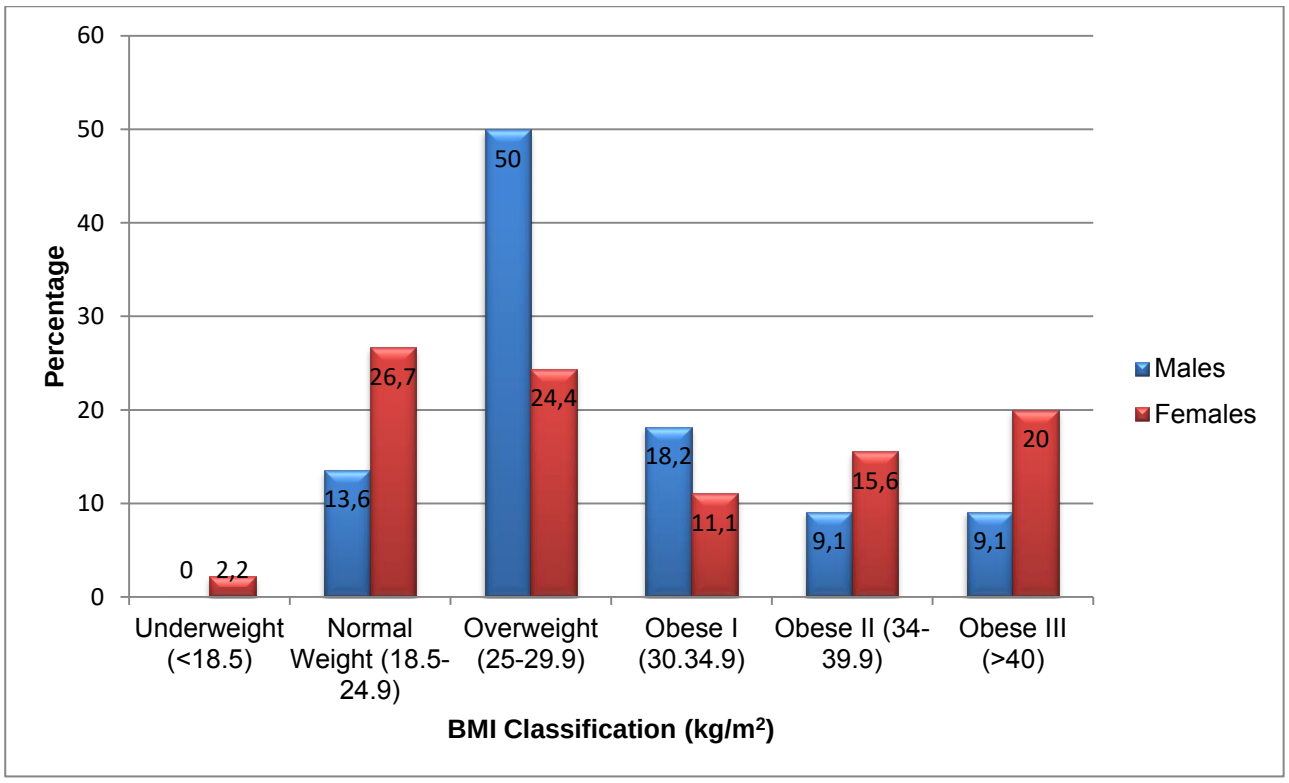
SD: Standard Deviation

Min: Minimum

Max: Maximum

BMI: Body Mass Index

The mean BMI of both the males and females fell in the obese category (30.5kg/m^2 (SD 6.1), with a minimum of 20.8kg/m^2 and maximum of 47.3kg/m^2 for males and 31.7kg/m^2 (SD 8.9), with a minimum of 18.0kg/m^2 and maximum of 51.8kg/m^2 for females. In this population, 86.4% of the males were overweight, of whom 36.4% were obese, and 71.1% of the females were overweight, of whom 46.7% were obese (Figure 3.2). Only 2.2% (*n*=1) of the females in the study population were underweight (BMI <18.5kg/m^2).



BMI : Body Mass Index

Figure 3.2: BMI Classification of the Study Population

Mean waist circumference of the males was 103.3cm (SD 14.6) and for the females, 94.9cm (SD 16.8). More than 40% (*n*=9) of the males had a waist circumference >102cm (substantially increased risk) and more than half (55.8%) (*n*=25) of the females had a waist circumference >88cm (substantially increased risk). Figure 3.3 depicts the spread of patients according to waist circumference category.

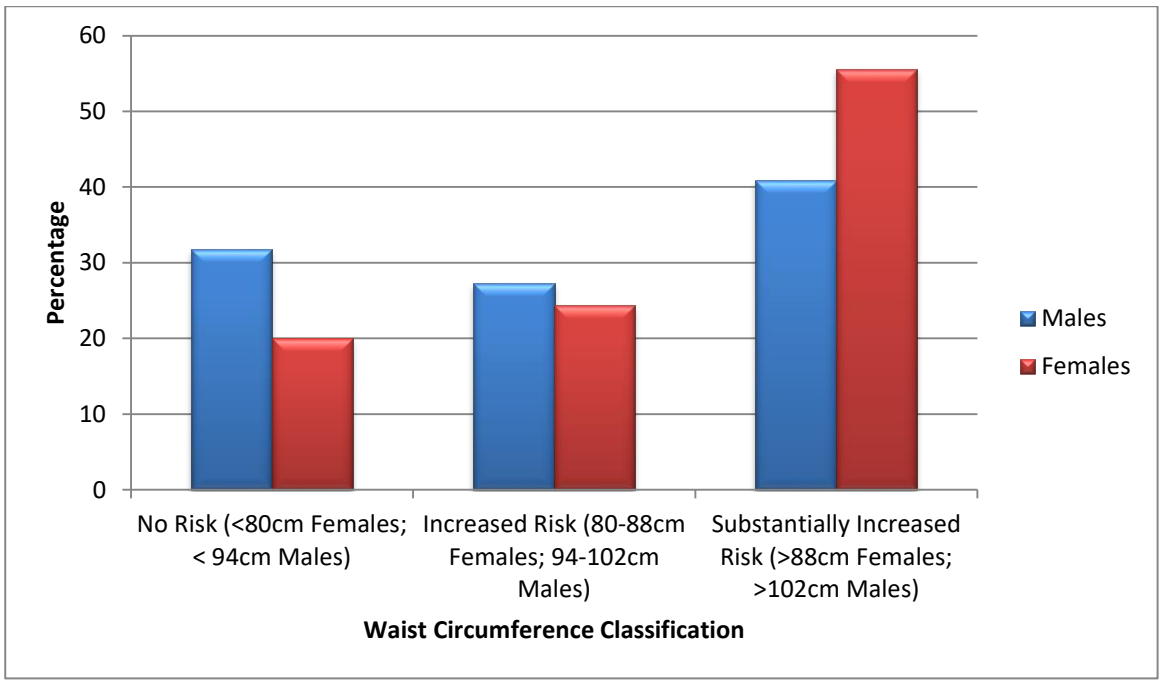


Figure 3.3: Waist Circumference Classification of the Study Population

3.1.5 **Skin-type classification of study population**

The skin-type classification of the study population according to the Fitzpatrick Skin Phototype Classification (Table 1.4) showed that the majority of the population were Type FZ II and Type FZ III (*n*=24, 35.8% and *n*=20, 29.9%) respectively, with FZ I and FZ VI having only *n*=1 (1.49%) each. Figure 3.4 depicts the spread of patients according to the Fitzpatrick Skin Phototype Classification.

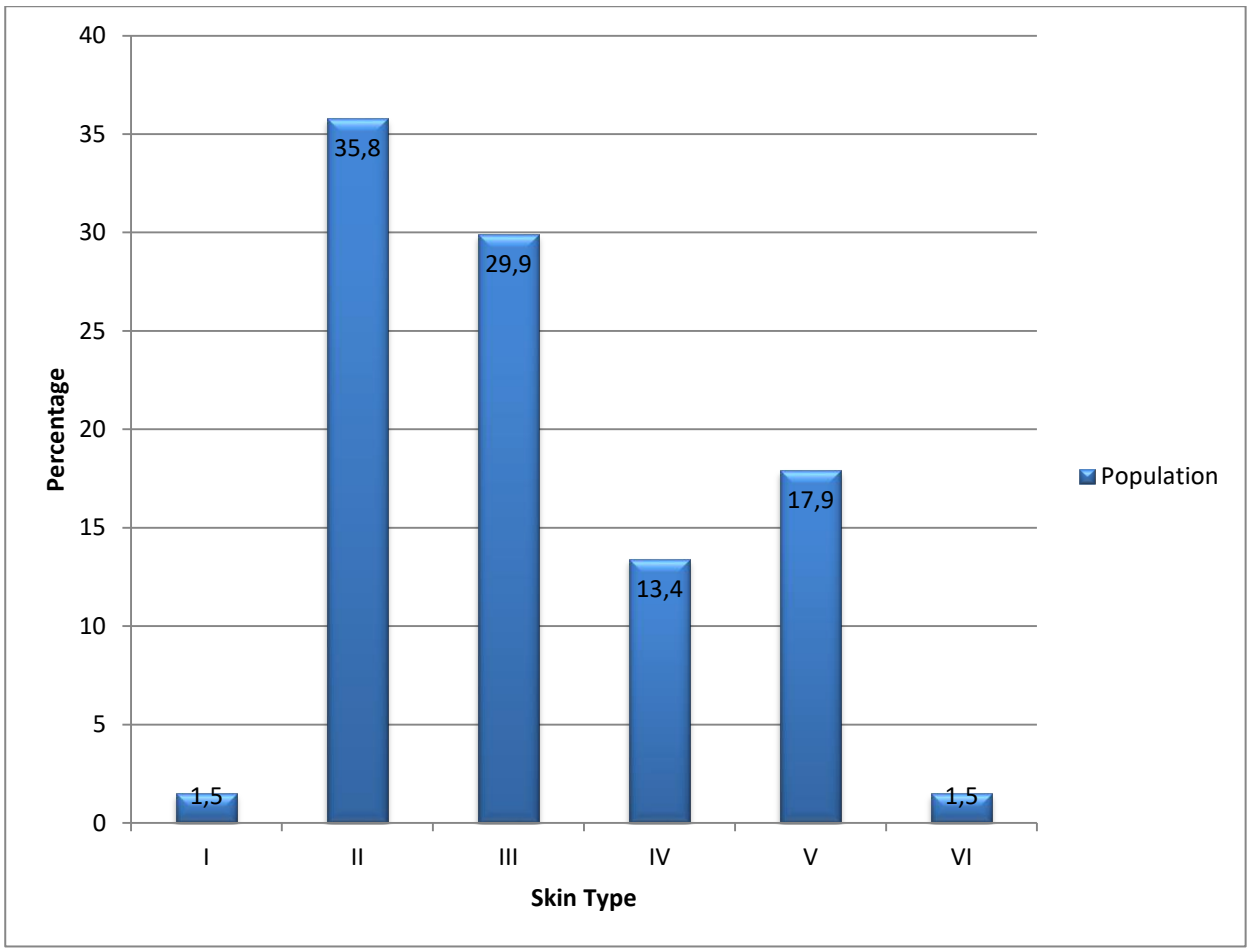
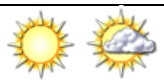
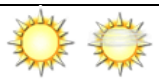
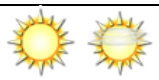
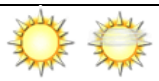


Figure 3.4: Fitzpatrick Skin Phototype Classification of the Study Population

3.1.6 **Sun exposure**

The average temperature in Johannesburg for the first period of data collection from April 2014 – September 2014 was 19ºC, with an average minimum temperature of 12ºC and maximum temperature of 25ºC.[122] (Table 3.4).

Table 3.5: Monthly Average Temperatures for Johannesburg April 2014–September 2014[122]

Month 2014	Weather Icons	Daily Average Temperature (ºC)	Minimum Temperature (ºC)	Maximum Temperature (ºC)
April		20	15	26
May		20	15	26
June		17	8	23
July		15	6	21
August		18	10	23
September		24	17	28
Average		19	12	25

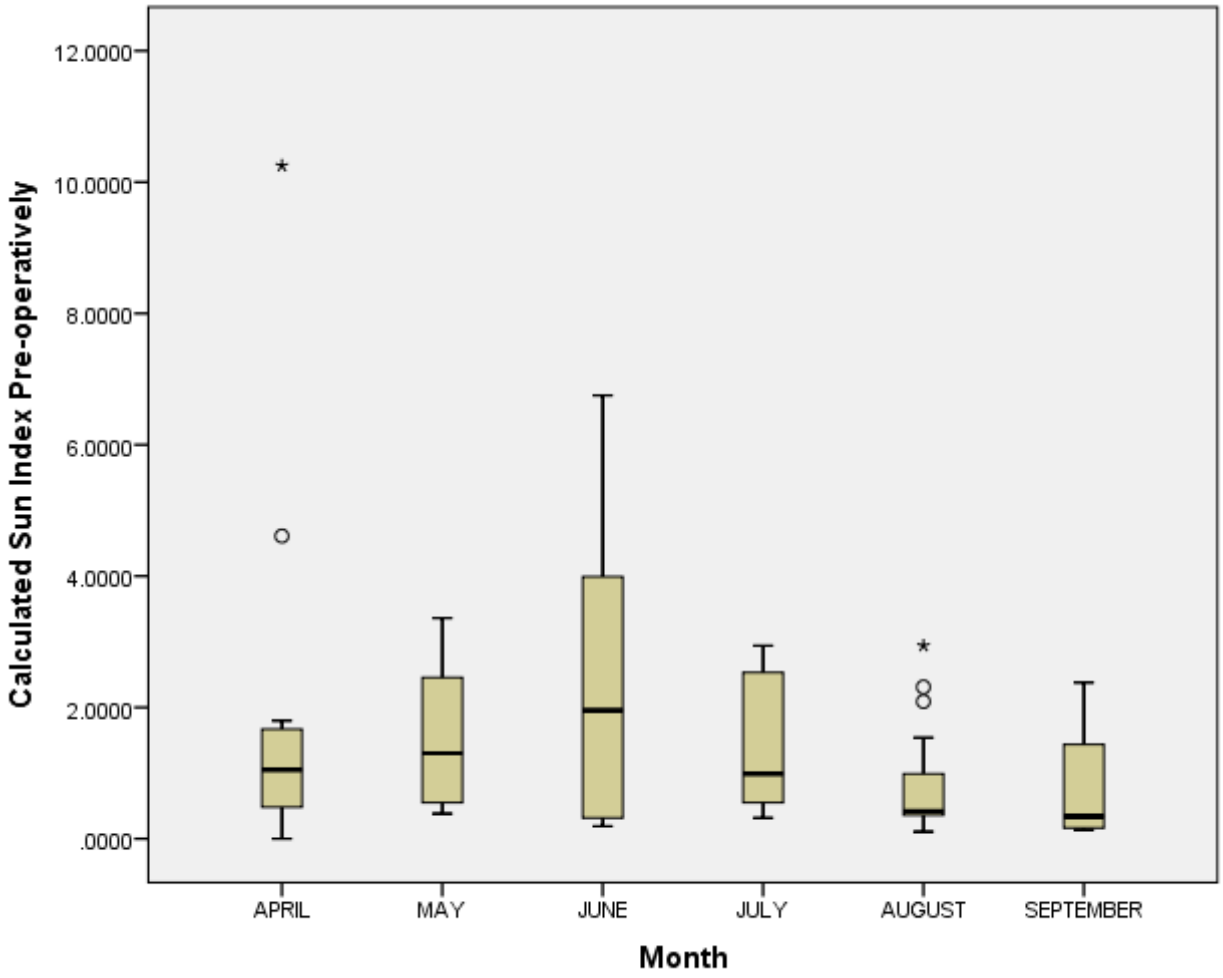
 *Daily, clear and sunny*

 *Daily, mainly clear and sunny*

 *Partly cloudy*

3.1.6.1 *Pre-operative sun exposure (calculated sun index)*

The median calculated sun index value of the pre-operative group (*n*=67) was 0.715; the 25th percentile value was 0.358 and the 75th percentile value was 2.075. The maximum value recorded was 10.25 and the minimum value was 0, owing to the use of sunblock and clothing. Sixteen patients were recruited in April, 8 in May, 13 in June, 5 in July, 21 in August and 4 in September. Sun exposure was similar during the months of May and July and was at its highest for the month of June. Lowest sun exposure was recorded during August (Figure 3.5).



**Very high calculated sun index of 10.25 in April*

ºHigh calculated sun index of 4.95 in April

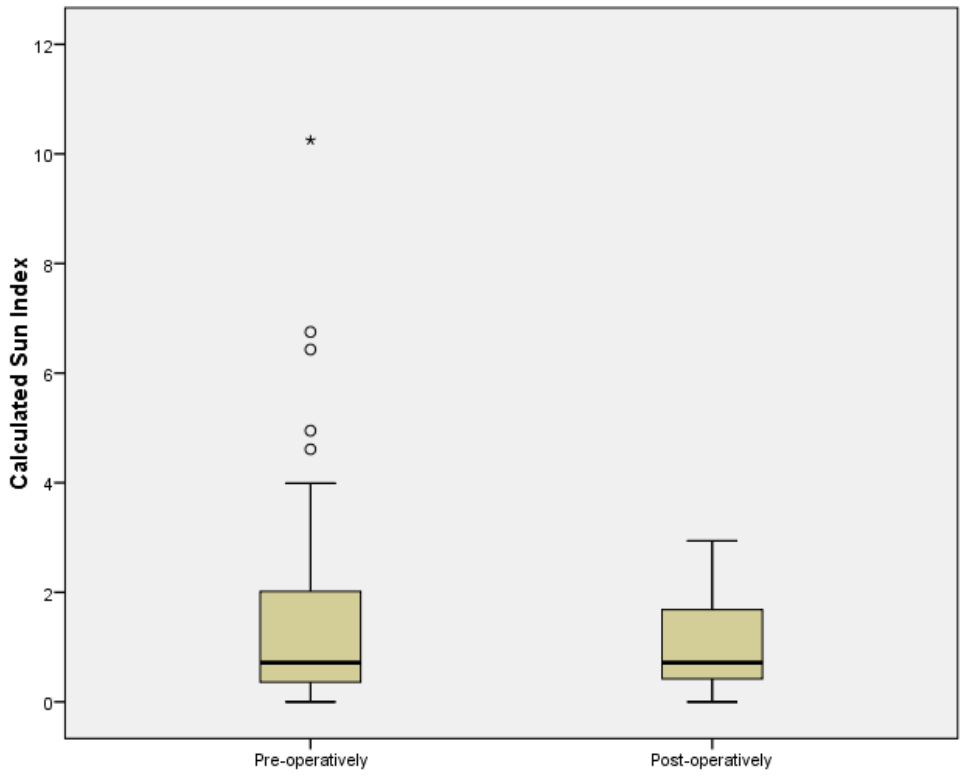
**High calculated sun index of 2.94 in August*

ºHigh calculated sun index of 2.31 and 2.09 in August

Figure 3.5: Calculated Sun Index over Data-Collection Period April 2014 to September 2014

3.1.6.2 *Post-operative sun exposure (calculated sun index)*

The median calculated sun index value was 0.715, the 25th percentile value was 0.385 and the 75th percentile value was 1.94. This indicates the same median sun exposure level in the post-operative group as in the pre-operative group, with slightly lower sun exposure on the 75th percentile. The maximum value recorded was 2.94 and the minimum value was 0. (Figure 3.6)



**Highest value*

ºHigh outlying values

Figure 3.6: Box Plot of Calculated Sun Exposure Indicated as Calculated Sun Index Pre-Operatively and Post-Operatively

3.1.7 **Vitamin D oral intake pre-operatively**

3.1.7.1 *Vitamin D supplementation intake*

Pre-operatively only 21% (*n*=14) of the study population took vitamin D supplementation. The median intake of the 14 subjects was 400 IU/day, with a minimum intake of 200 IU/day and a maximum intake of 1000 IU/day.

3.1.7.2 *Vitamin D food intake*

Pre-operatively the median oral daily intake of vitamin D from food was 195 IU, with a minimum intake of 24 IU and a maximum intake of 511 IU. With supplementation added to food intake, the daily values increase marginally to 202 IU(Table 3.5).

Table 3.6: Vitamin D Intake (IU) Pre-Operatively

Vitamin D intake: Oral and Supplementation IU (International Units)				
		Pre-operatively (*n*=67)		
		Oral Intake (*n*=67)	**Supplement (IU) (*n*=14 of 64)**	**Total Oral Intake (IU) (*n*=67)**
Minimum		24	200	24
Maximum		511	1000	1227
Percentiles	**25**	142	400	142
	50	195	400	202
	75	266	400	284

The Vitamin D intake from individual food sources ranged from 0 IU (cod liver oil and milk powder) to 109.8 IU (egg, whole) pre-operatively and 0 IU (cod liver oil) to 125.8 IU (egg, whole) post-operatively. Egg in both cases was the major source of vitamin D intake (Figure3.7).

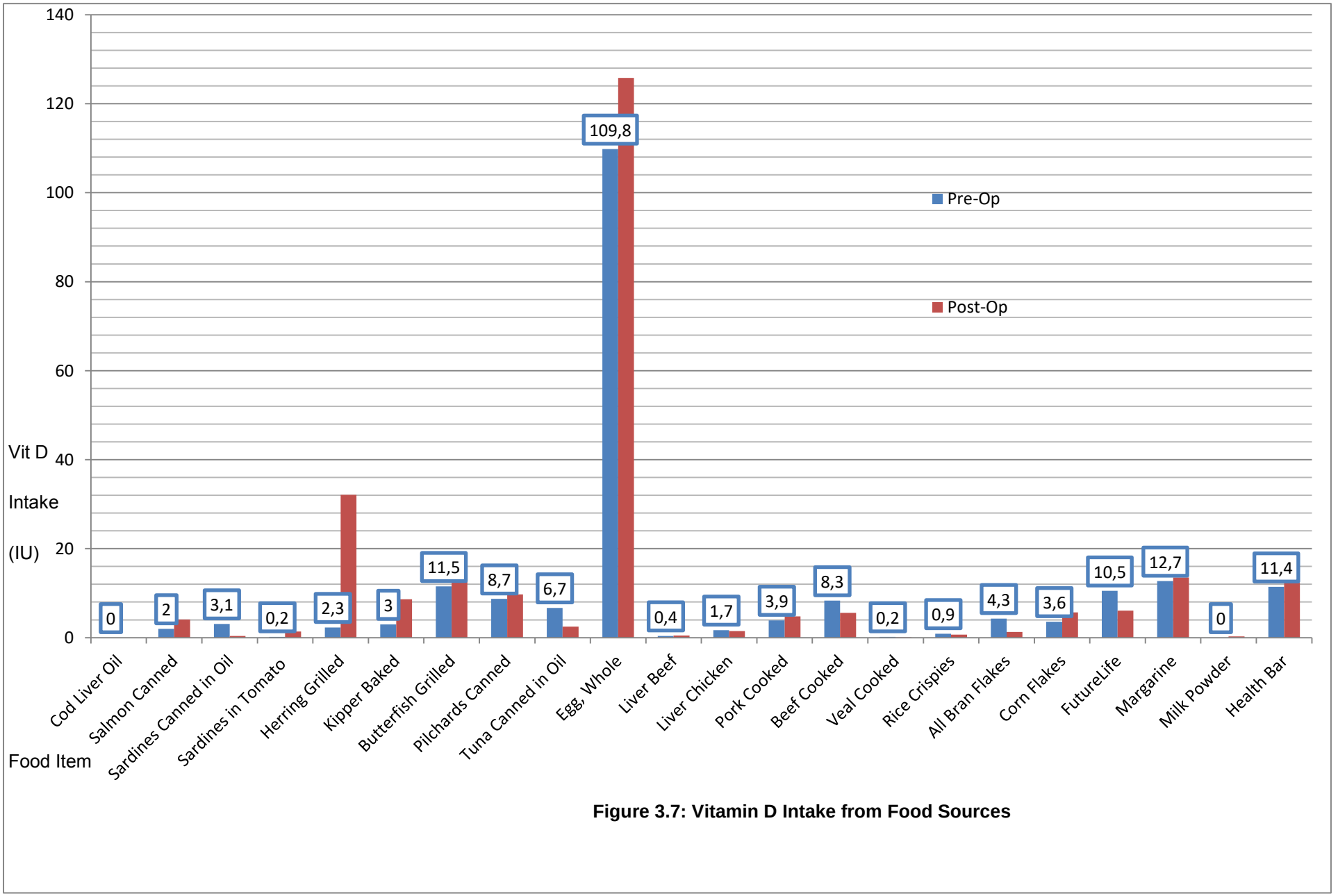


Figure 3.7: Vitamin D Intake from Food Sources

3.1.8 **Vitamin D status**

3.1.8.1 *Vitamin D status pre-operatively*

The mean vitamin D status (25(OH)D) of the study population was insufficient at 26.0ng/mL (SD 9.6), with a minimum of 10.0ng/mL and a maximum of 54.0ng/mL. Thirty-five point eight percent (*n*=24) had insufficient vitamin D levels and 29.9% (*n*=20) had deficient vitamin D levels; this means that 65.7% (*n*=44) did not have sufficient (>30ng/mL) vitamin D status. Only 34.3% (*n*=23) of the study population had sufficient levels of vitamin D(Figure 3.8). The average 25(OH)D values according to season for the warmer months (April, May, September) were 28.0ng/mL (insufficient) and for the colder months (June, July August) 26.4ng/mL (insufficient).

When using the IOM cut off values for vitamin D status 70% (*n*=47) had sufficient (>20ng/mL) vitamin D status, 28% (*n*=19) had insufficient (12-20ng/mL) vitamin D status and 2% (*n*=1) had deficient (<12ng/mL) vitamin D status.

3.1.8.2 *Vitamin D status post-operatively*

The mean vitamin D status of the study population was insufficient at 28.2ng/mL (SD 10.2), with minimum and maximum levels of 8.4ng/mL and 50ng/mL respectively. Thirty-nine point one percent (*n*=9) had sufficient vitamin D levels, while 39.1% (*n*=9) had insufficient vitamin D levels and 21.7% (*n*=5) had deficient levels. This means that 60.9% (*n*=14) did not have sufficient (>30ng/mL) vitamin D status (Figure 3.8). The average 25(OH)D values according to season for the warmer months were 28.0ng/mL (insufficient) and for the colder months 28.3ng/mL (insufficient).

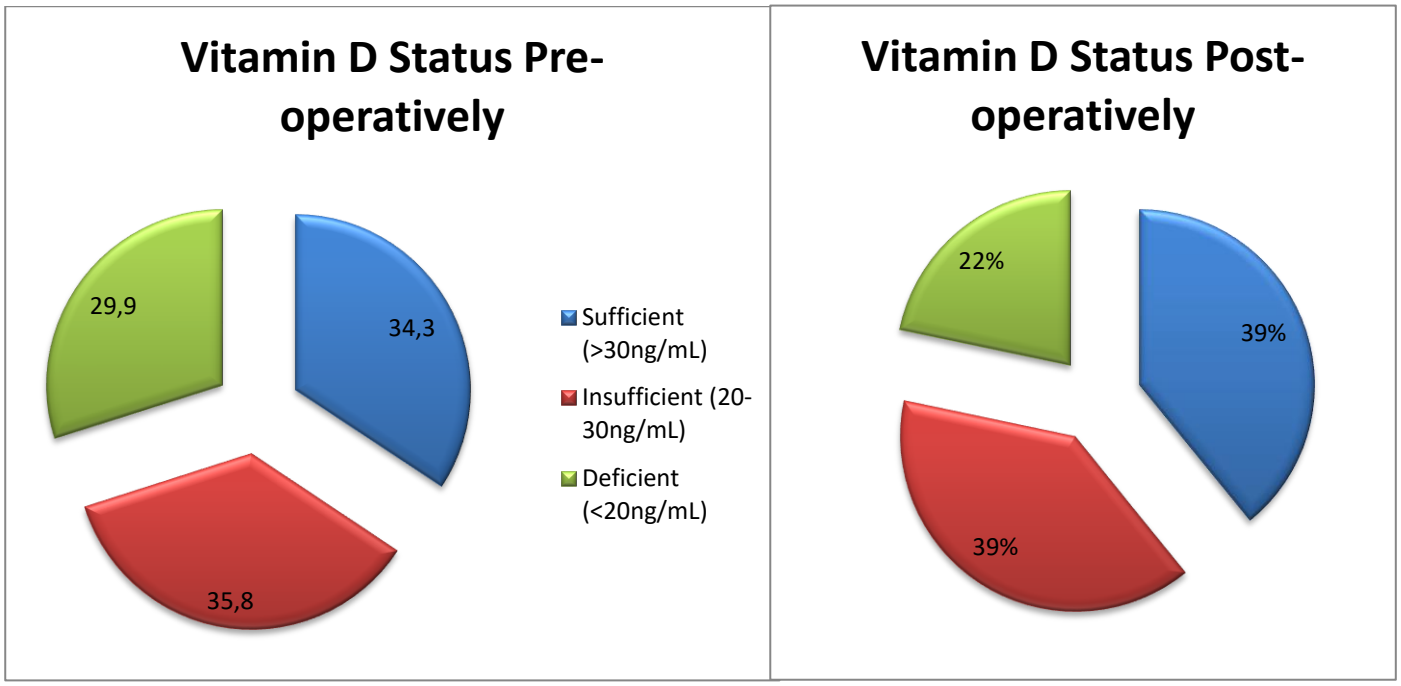
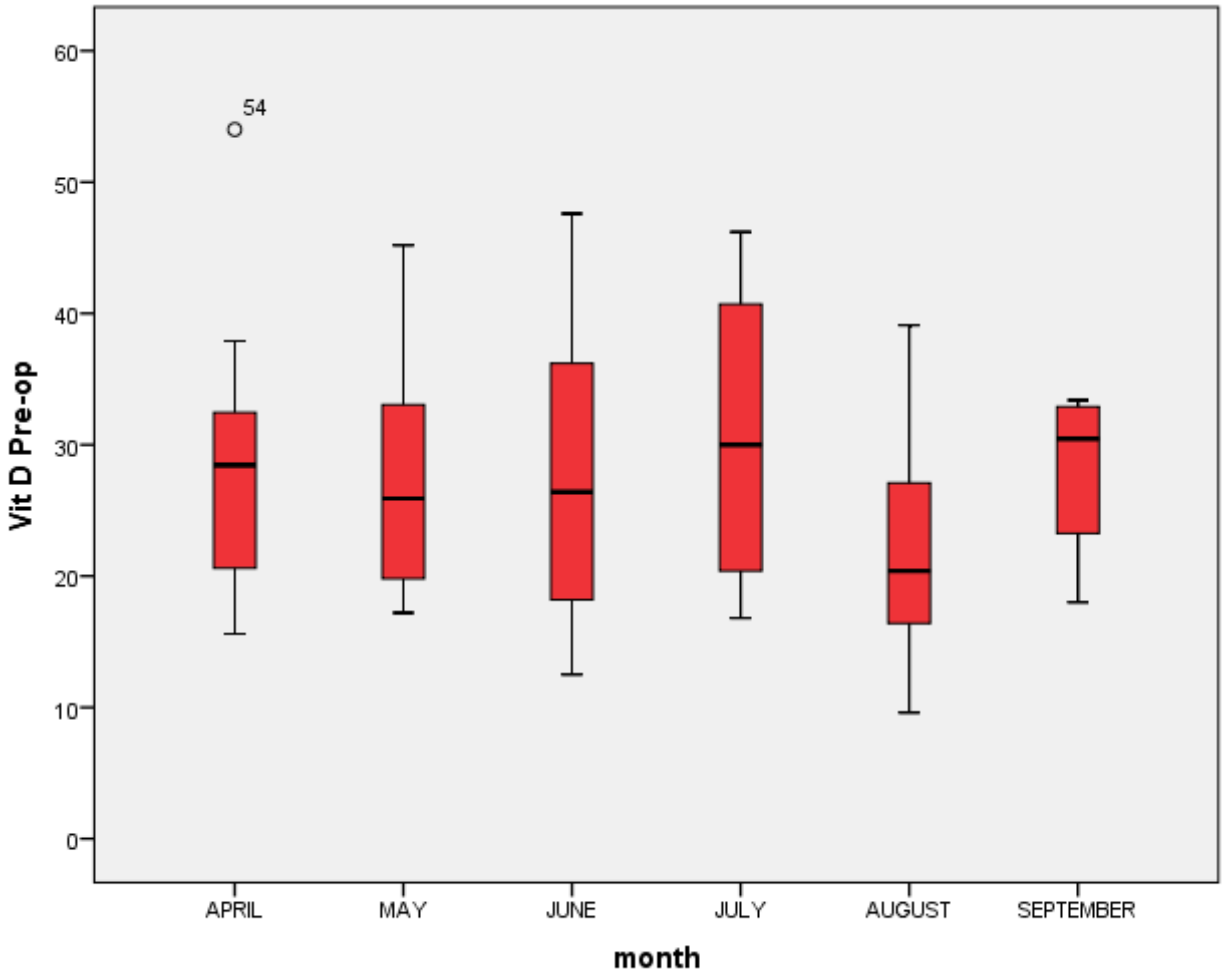


Figure 3.8: Vitamin D Status Pre- and Post-Operatively

Median 25(OH)D values (pre-operatively) for July peaked at 30ng/mL and then decreased in August (20ng/mL), increasing again in September to 30ng/mL (Figure 3.9).



[9]Highest value

Figure 3.9: 25(OH)D over the Data-Collection Period of April 2014–September 2014

3.2 Relationship between 25(OH)D and Parameters Pre-Operatively

3.2.1 The relationship between 25(OH)D and the demographic parameters pre-operatively

3.2.1.1 *Age (pre-operatively)*

The relationship between age and 25(OH)D pre-operatively indicated a weak positive correlation coefficient according to Pearson's correlation of r=0.249.This value is statistically significant (p=0.042). Linear regression (R^2=0.062) indicates age as a weak predictor of 25(OH)D in this population group (Figure 3.10).

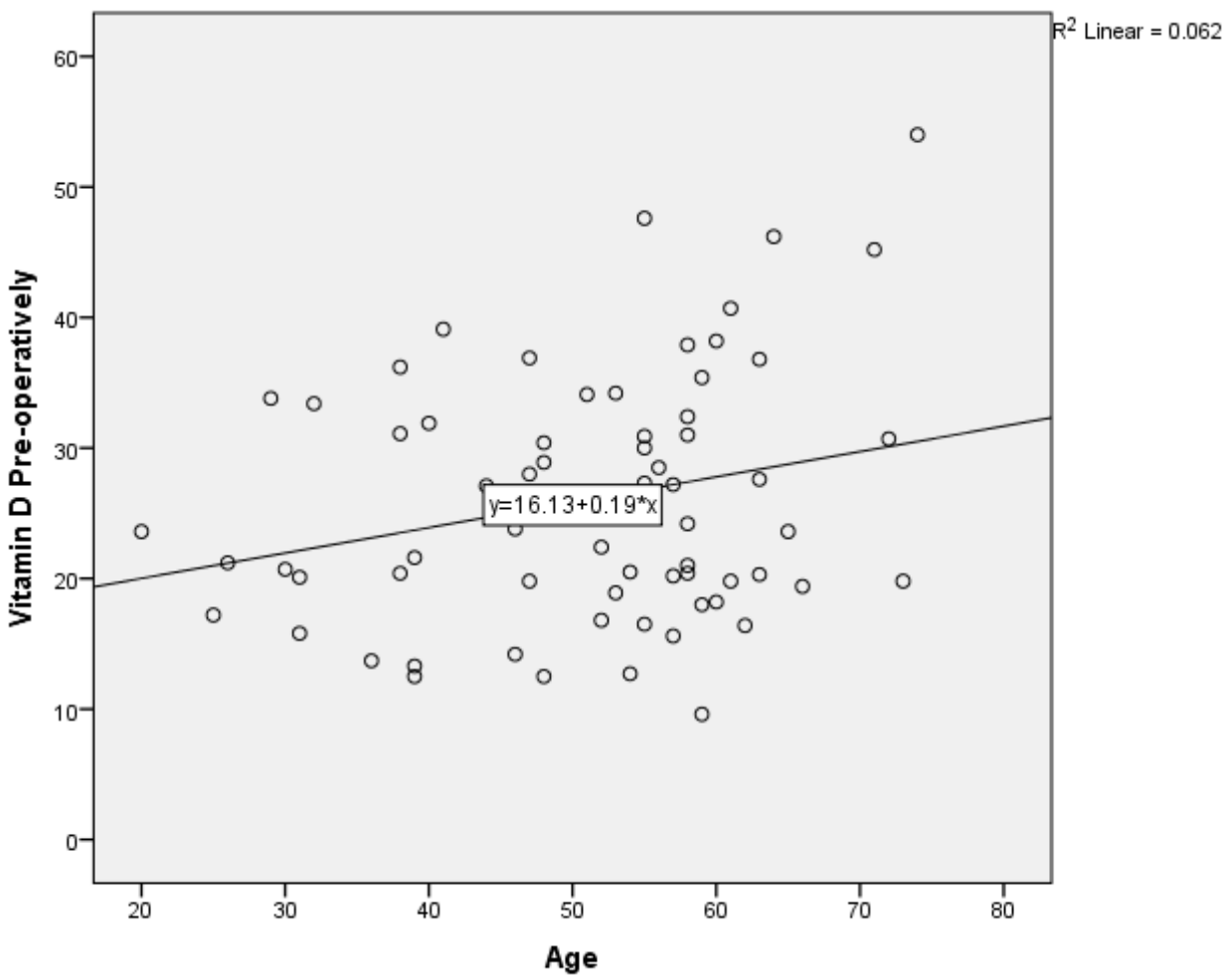


Figure 3.10: 25(OH)D Pre-Operatively Correlated for Age

3.2.1.2 *Gender (pre-operatively)*

Pre-operative mean 25(OH)D for males (29.2ng/mL) was higher than that for females (24.5ng/mL), although not significantly so (p=0.057; *t-test*).

3.2.1.3 *Smoking (pre-operatively)*

Pre-operatively the smokers had a mean vitamin D value of 22.8ng/mL versus 27.04ng/mL for the non-smokers. The mean difference was -4.2ng/mL. This difference was not significant (p=0.126; *t-test*).

3.2.1.4 *Skin classification (pre-operatively)*

Mean 25(OH)D as per the Fitzpatrick Skin Phototype Classification with minimum and maximum levels is summarised in Table 3.6. The mean values ranged from 15.6ng/mL (Type FZ VI) to 30.1ng/mL (Type FZ II).

Table 3.7: Summery of 25(OH)D as per Skin Classification Pre-Operatively

Fitzpatrick Skin Phototype Classification	*N*	Mean (ng/mL)	Standard Deviation (SD)	95% Confidence Interval for Mean (ng/mL)		Minimum (ng/mL)	Maximum (ng/mL)
				Lower Bound	Upper Bound		
Type FZ I	1	16.4	.	.	.	16	16
Type FZ II	24	27.6	10.5	23.2	32.0	13	54
Type FZ III	20	30.1	9.3	25.7	34.5	10	46
Type FZ IV	9	22.6	7.7	16.7	28.6	13	32
Type FZ V	12	20.3	5.6	16.7	23.9	13	34
Type FZ VI	1	15.6	.	.	.	16	16
Total	67	26.0	9.6	23.7	28.4	10	54

One-way ANOVA was used to compare 25(OH)D levels of four different skin types (II – V). Skin types I and VI were omitted as they only had one subject per group. In the investigation to determine significant differences between the different groups, it was found that there was only a significant difference between group III and V (p=0.025). The mean difference was 9.8ng/mL, with group III having the highest values and group V having the lowest values. Mean 25(OH)D as per skin type pre- and post-operatively is illustrated in Figure 3.11.

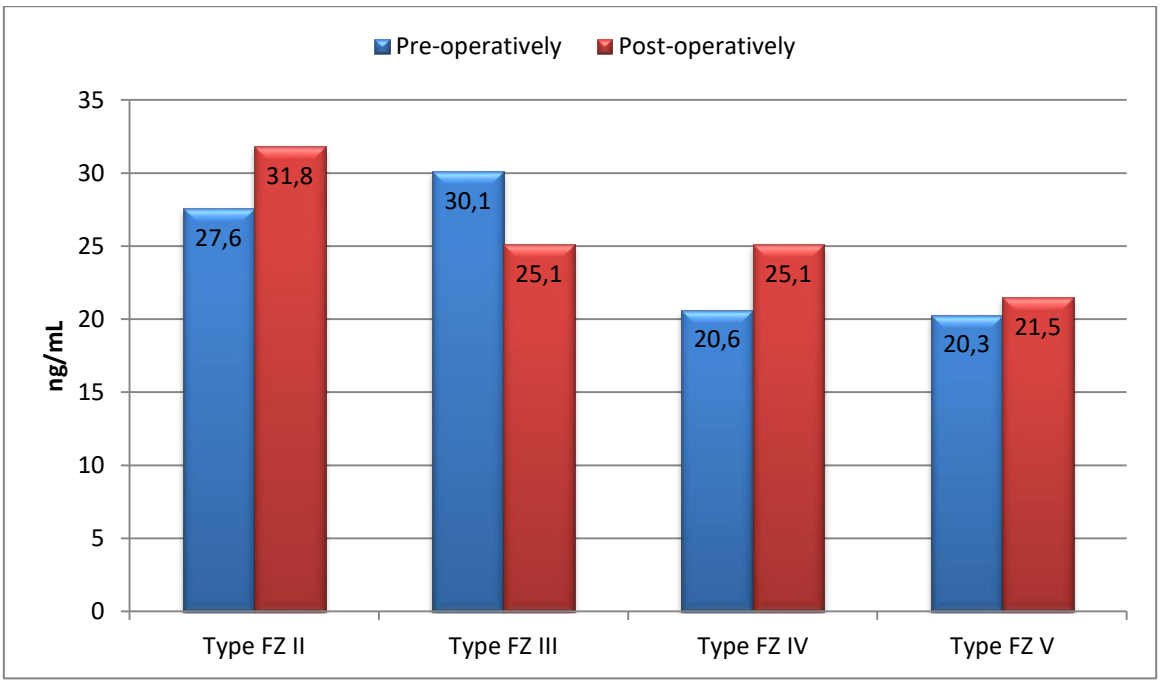


Figure 3.11: Mean 25(OH)D correlated with The Fitzpatrick Skin Phototype Classification; Skin Type II to V

3.2.2 Relationship between 25(OH)D and anthropometrics pre-operatively

The Pearson correlation coefficient between 25(OH)D and BMI was r=0.042 and between 25(OH)D and waist circumference r=0.112. There was no correlation between 25(OH)D and BMI (p=0.733) and waist circumference (p=0.365).

3.2.3 Relationship between 25(OH)D and sun exposure (calculated sun index) pre-operatively

The Spearman's rank correlation coefficient between 25(OH)D and the calculated sun index was r=0.451, which indicates a moderate positive correlation (p<0.001). Linear regression (R^2=0.103) indicates calculated sun index as a weak predictor of vitamin D status in this population group (Figure 3.12).

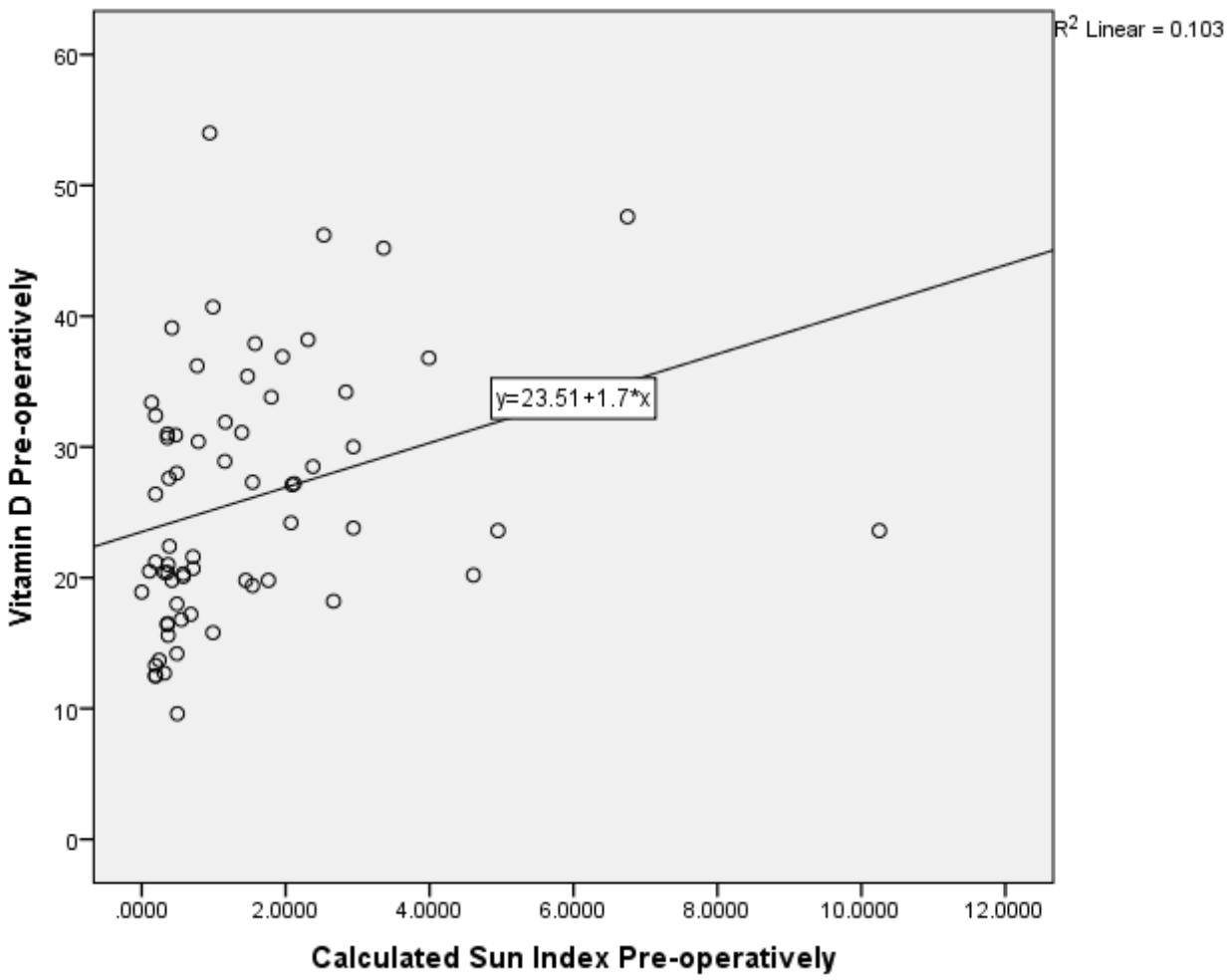


Figure 3.12: 25(OH)D Pre-Operatively Correlated for Calculated Sun Index Pre-Operatively

3.2.4 **Relationship between 25(OH)D status and vitamin D intake pre-operatively**

The Spearman's rank correlation coefficient between 25(OH)D and oral vitamin D intake was r=0.066 (p=0.598), for vitamin D supplementation, r =0.087 (p=0.483), and for total vitamin D intake, r=0.097 (p=0.434), indicating no correlation between 25(OH)D and oral vitamin D intake.

3.3 Relationship between 25(OH)D and Parameters Post-Operatively

3.3.1 The relationship between 25(OH)D and the demographic parameters post-operatively

3.3.1.1 *Age (post-operatively)*

The correlation coefficient according to Pearson's correlation post-operatively was r=0.194 (p=0.375), and was not statistically significant.

3.3.1.2 *Gender (post-operatively)*

Post-operatively the mean 25(OH)D for males was 33ng/ml and for females 27.1ng/ml. This difference was not statistically significant (p=0.316; t-test).

3.3.1.3 *Smoking (post-operatively)*

Using the One-way ANOVA test, there was no difference between smokers (n=2) and non-smokers (n=21) post-operatively (p=0.602).

3.3.1.4 *Skin classification (post-operatively)*

Mean 25(OH)D ranged from 15.8ng/mL (Type FZ VI) to 31.8ng/mL (Type FZ II). Mean 25(OH)D as per the Fitzpatrick Skin Photopype Classification is summarised in Table 3.7. With One-way ANOVA testing there was no statistical difference between the different skin tones post-operatively (n=23) (p=0.389).

Table 3.8: Summary of 25(OH)D Post-Operatively

Fitzpatrick Skin Phototype Classification	25(OH)D Post-operatively (ng/mL)				
	Valid *n*	Mean	Standard Deviation (SD)	Minimum	Maximum
Type FZ I	0	-	-	-	-
Type FZ II	13	31.8	9.8	15.7	51.6
Type FZ III	6	25.1	11.2	8.4	36.3
Type FZ IV	1	25.1	-	25.1	25.1
Type FZ V	2	21.5	5.2	17.8	25.1
Type FZ VI	1	15.8	-	15.8	15.8

3.3.2 Relationship between 25(OH)D and anthropometrics post-operatively

The Spearman's rank correlation coefficient between 25(OH)D post-operatively and BMI was r=0.141 and for waist circumference r=0.045. There was no correlation between 25(OH)D and anthropometrics post-operatively.

3.3.3 Relationship between 25(OH)D and sun exposure (calculated sun index) post-operatively

The Spearman's rank correlation coefficient between 25(OH)D and the calculated sun index was r=0.397, but this was not statistically significant compared with the post-operatively calculated sun index (p=0.413).

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3.3.4 Relationship between 25(OH)D and vitamin D intake post-operatively

The Spearman's rank correlation coefficient between 25(OH)D and oral vitamin D intake was r=0.066, for vitamin D supplementation r = 0.087 and for total vitamin D intake r=0.097, indicating no correlation between 25(OH)D and oral vitamin D intake.

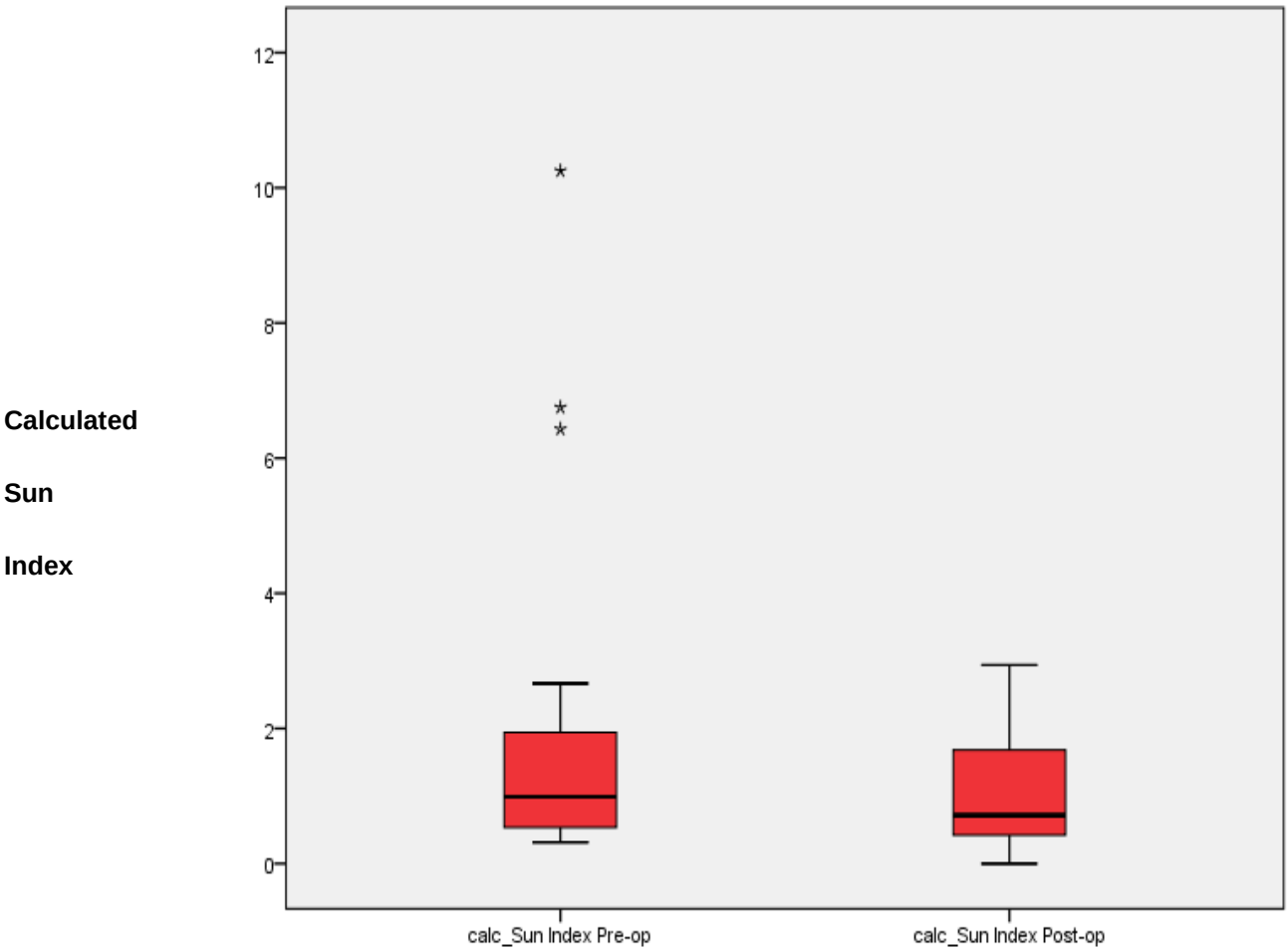
3.4 Pre-Operative Parameters in Relation to Post-Operative Parameters

Twenty-three of 67 (34%) subjects returned for follow-up visits. Follow-up procedures were discussed in Section 2.4.1.

3.4.1 Sun exposure (calculated sun index) pre-operatively and post-operatively

The median sun exposure (calculated sun index) value for the pre-operative group that came for follow-up (n=23) and for the post-operative group (n=23) was 0.99 and 0.715, respectively, with a minimum value for the pre-operative group of 0.315 and a maximum value of 10.25. Minimum value post-operatively was 0 and maximum value 2.94 (Figure 3.13). There was no significant difference between pre- and post-operative calculated sun index values (p=0.144).

Retain null hypothesis: There is no statistically significant change in sun exposure (calculated sun index) after orthopaedic surgical intervention when compared with the pre-surgery status.



**High outlying values*

Figure 3.13: Calculated Sun Index of Pre-Operative (*n*=23) Group for Follow-Up and Post-Operative Group (*n*=23)

3.4.2 Vitamin D intake pre-operatively and post-operatively

Median values for oral intake of vitamin D from food for the pre-operative group (*n*=23) was 226 IU, with a minimum and maximum intake of 64 IU and 454 IU. The 25th and 75th interquartile range values were 168 IU and 344 IU respectively. When total intake (supplementation and food) is calculated, the values increase marginally to 327 IU for the 50th (median) percentile and 685 IU for the 75th percentile (Figure 3.14).

The median oral intake of vitamin D from food for the post-operative group (*n*=23) was 240 IU, with a minimum intake of 67 IU and a maximum oral intake of 669 IU. The 25th and 75th interquartile ranges were 101 IU and 354 IU respectively. With supplementation added to food intake, the values increase marginally to 340 IU for the 50th (median) percentile and

639 IU for the 75th percentile. There is no significant difference in vitamin D intake between the pre-operative and post-operative group (p=0.465)

Retain null hypothesis: There is no statistically significant change in vitamin D intake after orthopaedic surgical intervention compared with the pre-surgery status.

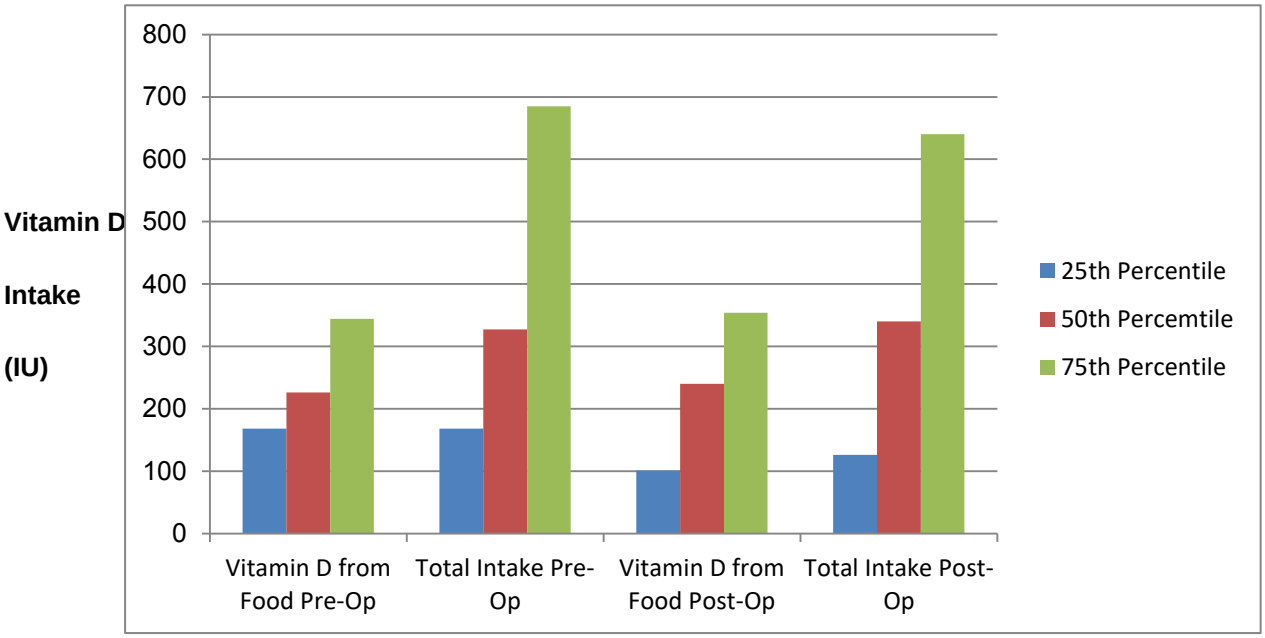


Figure 3.14: Vitamin D Intake Pre-Operatively (n=23) and Post-Operatively (n=23)

3.4.3 **25(OH)D pre-operatively and post-operatively**

The mean 25(OH)D of the pre-operative group was 28.7ng/ml (SD 11.6ng/mL) and for the post-operative group, 28.1ng/mL (SD 10.2ng/mL).

The mean paired difference of the two groups was 0.48 (SD 5.03). There is no significant difference in 25(OH)D between pre-operative and post-operative groups (p=0.656). Using chi-square tests, there is no significant change in 25(OH)D status from the pre-operative to the post-operative state (p=0.549).

Retain null hypothesis: There is no statistically significant change in 25(OH)D after the orthopaedic surgical intervention period compared with the pre-surgery status.

3.5 Calculated Sun Index Values and Vitamin D Status

The clinical recommendation for sun exposure is 15–30 minutes of sun exposure on arms and legs 2–3 times per week. Sun exposure time per week = 1.5 hours. BSA (Body Surface Area): 0.14 (arms) plus 0.24 (legs) = 0.38 BSA. The calculated sun index for the clinical recommendation: 1.5 X 0.38 = 0.615.

Pre-operatively 46.3% (*n*=31) of subjects had a calculated sun index value below the recommended value of 0.615; of these 19.4% (*n*=6) had sufficient vitamin D status and 80.6% (*n*=25) had insufficient and deficient vitamin D status. Of the 53.7% (*n*=36) of subjects that had calculated sun index values above the recommended value of 0.615, 52.8% (*n*=19) had insufficient and deficient vitamin D status (Table 3.9).

Table 3.9:Cross-Tabulation of Vitamin D Status and Calculated Sun Index Pre-Operatively

Vitamin D Status		Calculated Sun Index		Total
		< 0.615	≥ 0.615	
Sufficient >30ng/mL	Count (*n*)	6	17	23
	%	19.4	47.2	34.3
Insufficient and Deficient ≤30ng/mL	Count (*n*)	25	19	44
	%	80.6	52.8	65.7
Total	Count (*n*)	31	36	67
	%	46.3	53.7	

Post-operatively 39.1% (*n*=9) of subjects had a calculated sun index value below the recommended value of 0.615; of these 22.2% (*n*=2) had sufficient vitamin D status, and 77.8% (*n*=7) had insufficient and deficient vitamin D status. Of the 60.9% (*n*=14) of subjects that had calculated sun index values above the recommended value of 0.615, 50% (*n*=7) had insufficient and deficient vitamin D status (Table 3.11).

Table 3.10:Cross-Tabulation of Vitamin D Status and Calculated Sun Index Post-Operatively

Vitamin D Status		Calculated Sun Index		Total
		< 0.615	≥ 0.615	
Sufficient >30ng/mL	Count (*n*)	2	7	9
	%	22.2	50	39.1
Insufficient and Deficient ≤30ng/mL	Count (*n*)	7	7	14
	%	77.8	50	65.7
Total	Count (*n*)	9	14	23
	%	39.1	60.9	

There is a statistically significant association between vitamin D levels and calculated sun index pre-operatively (p=0.017). One is more likely to have sufficient vitamin D levels if one has a sun exposure index of > 0.615, but almost equally likely to be insufficient or deficient if one's index is > or < than the recommended levels of 0.615. Interpretation for post-operative correlation is the same as for pre-operative, but not statistically significant (p=0.288).

3.6 Predicted Calculated Sun Index Levels for Sufficient 25(OH)D

Using the receiver operating characteristic (ROC) curve to determine sensitivity and specificity of the calculated sun index value to predict sufficient 25(OH)D, the recommended sun index value of 1.159 yielded sensitivity of 68.2% and specificity of 56.5% (Figure 3.15). The area under the curve was 0.644 (p=0.054).

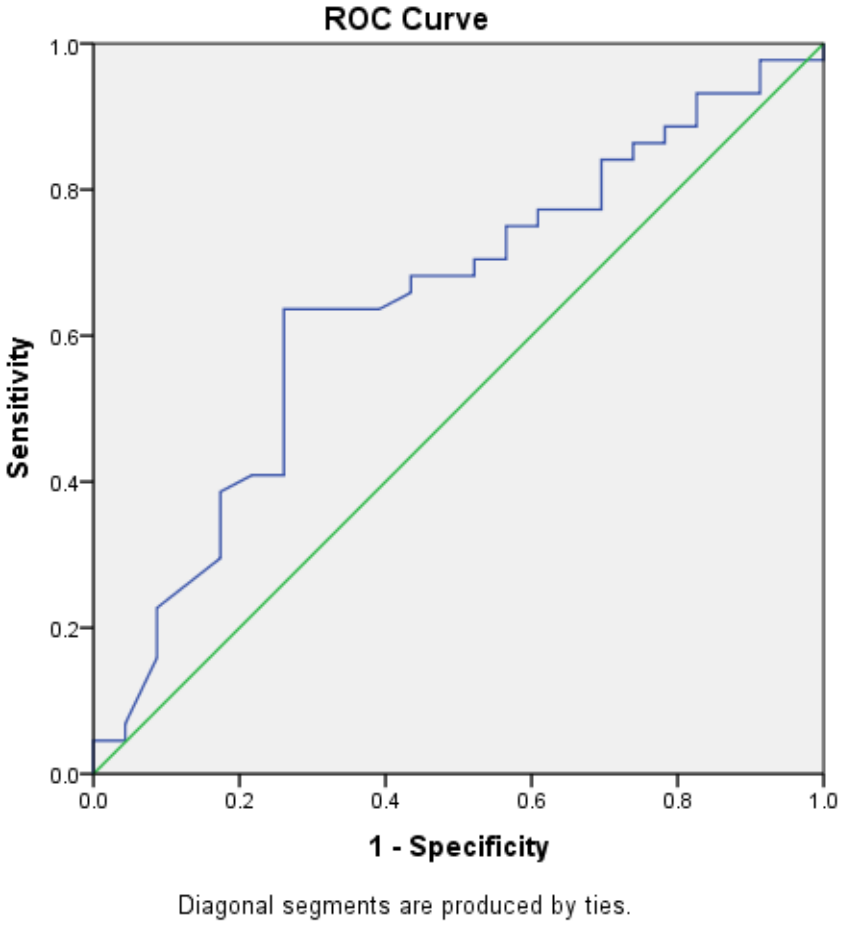


Figure 3.15: ROC Curve for Calculated Sun Index and 25(OH)D

CHAPTER 4: DISCUSSION

4 General

It is well known that vitamin D is related to the prevention of rickets in children, but it is also critically important in the maintenance of calcium and bone metabolism throughout the life cycle. For adults, vitamin D deficiency can have subtle but important consequences for the musculoskeletal system.[26] To the author's knowledge, this is the first study of its kind done in South Africa investigating the vitamin D status of adult surgical orthopaedic patients in a private setting. Vitamin D status prevalence studies in South Africa in the adult population are limited. In this study, the parameters investigated were anthropometrics, sun exposure and vitamin D intake, and their relationship to vitamin D status pre- and post-operatively.

4.1 Study Population Demographics

A period of seven months was needed to recruit the 67 patients required for the study. The present study included adult females and males, where the majority of the group were younger than 65 years. Similar vitamin D prevalence studies have included only females,[33,51] one study only males,[86] and other studies, both genders.[15,25] In the comprehensive review on worldwide status of vitamin D nutrition by Van Schoor and Lips, the age range of the populations varied from 18 to 85 years.[25] This study represents a heterogeneous population including adult patients from various age groups, ethnic groups, language groups and a variety of minor and major elective orthopaedic surgeries.

The two most prevalent chronic conditions in the population studied were hypercholesterolemia and hypertension. The chronic medications used in the treatment of these chronic conditions are not documented to have an impact on vitamin D status. A variety of medications, including anticonvulsants and medications to treat HIV/AIDS, can put patients at risk of vitamin D deficiency because these drugs enhance the catabolism of 25(OH)D and $1,25(OH)_2D$.[98] These patients were not included in the study population.

Almost a quarter of this study population were current smokers, which accords with the figures released by the Medical Research Council (MRC), where the prevalence of adult daily cigarette smoking in South Africa was recorded at 24.1% in 2004.[123] More recent results published by the first South African National Health and Nutrition Examination Survey (SANHANES-1)(2013) reported current smoking by South African adults as 18.2%,

marginally lower than this study population.[124] Smoking status in this study population did not seem to influence vitamin D status.

In this study the mean weight of females (84.0kg) and males (94.0kg) was higher than the national average as reported by SANHANES-1 (72.2kg and 67.3kg respectively).[124] The highest mean weights reported by SANHANES-1 were in the age groups 45–54 and 55–64 for both genders (74.7kg and 71.8kg for males and 79.4kg and 77.2kg for females respectively).[124] As the mean age of this study population was 50.9 years, the latter data is more comparable.

In the analysis of worldwide mean BMI trends in populations from 1980 to 2008, the data showed an increase of between 0.4 and 0.5 kg/m^2 per decade for males and females. Females from South Africa maintained their position in the top four BMI sub-regions (North America, North Africa and Middle East, and Southern Africa) compared with 1980.[125] The current SANHANES-1 survey shows that the trend for increasing mean BMI in South Africa has continued, particularly among females.[124] The mean BMI of this study for females (31.7kg/m²) and males (30.5kg/m²) was much higher than the SANHANES-1 data of South Africans overall (28.9kg/m² and 23.6kg/m² respectively).[124] In South African females (SANHANES-1) the prevalence of overweight (24.8%) is similar to this study (24.4%), but the obesity rate (39.2%) is much higher in this study (46.7%). However the males in this study have much higher overweight (50%) and obesity (36.4%) rates than the average South African (20.1% and 10.6% respectively). In the SANHANES-1 data, the BMI of the older age groups was significantly higher for both females and males. The age groups 45–54 years, 55–64 years and 65 years and older had mean BMIs (31.7kg/m^2, 31.3kg/m^2, 30.0kg/m^2 for females, respectively, and 26.0kg/m^2, 25.2kg/m^2, 25.6kg/m^2 for males, respectively).[124] The average BMI classification of this study was obese class I (>30kg/m²); however the average BMI of the participants in other studies on vitamin D status done in the United Kingdom,[98] Massachusetts in the United States,[33] and Hawaii,[15] was all within normal range (18.5–24.9kg/m²). The typical orthopaedic surgical patient in 26 hospitals across America, according to Tedeseco et al., was 65.5 (±14.5) years old. Of these, 20.2% (SD 15.4%) were obese.[126] In a Swiss study, Zingg et al. noted that the number of patients undergoing orthopaedic surgeries, in this case total knee arthroplasty, was disproportionately high, with more than 42% of patients being obese[127]. These figures are more in line with the average obesity rates of this study (46.7%).

Overweight and obese patients have more surgical and post-operative problems and complications than patients with normal BMIs. Obese patients are also further more likely to experience micro-trauma injuries to their upper limbs than patients with a normal BMI, primarily because of motor control problems.[128] A clear link has been established between osteoarthritis and obesity, owing to excess mechanical loads and the biological effects on the cartilage. The obesity effect is more apparent in the knee than the hip. A person with a BMI > 30 kg/m^2 is 8.5 times more likely to need a joint replacement than someone with a normal BMI; this risk will increase to 18.7 times if the BMI is over 35kg/m² and to 32.7 times if the BMI is over 40kg/m². Also, obese patients are operated on an average of ten years earlier than their normal BMI counterparts.[128] The over-representation of overweight individuals among patients requiring general orthopaedic elective surgeries has also been seen in the study done by Böstman, where the population was 22–27% obese, compared with the general population of 16%.[129] The correlation between BMI and 25(OH)D is further explored later.

A population level mean waist circumference that exceeds the recommended waist circumference cut-off (equal to or more than 102 cm in males and 88 cm in females) is indicative of a substantially increased risk of metabolic complications.[121] According to SANHANES-1 data, the mean waist circumference for males (81.4cm) and females (89cm) was lower than the average of this study (103.3cm and 94.9cm respectively). SANHANES-1 data indicate that 10% of males had a waist circumference ≥ 102 cm, while approximately half of females had a waist circumference ≥ 88 cm. This is much lower than the data from this study (40.9% and 57.8% respectively). The review article by Parratte et al. on obesity in orthopaedic and trauma surgery does not include any data on waist circumference. BMI is the most common indicator for obesity used in the research.[126-128.]

The Fitzpatrick Skin Phototype Classification used in this study has been validated[87,89] and used in numerous studies.[2,87-91] Other studies investigating vitamin D status in adults have not taken into account skin classification[33] and/or ethnic group.[114] Another study used reflectance colourimetry, where the Commission Internationalede l'Eclairage L scale was used that ranges from 0 (black) to100 (white) and represents a system created by the International Commission on Illumination to accurately represent human colour perception.[15]

4.2 Sun Exposure

South Africa is a country on the most southern tip of the African continent, with coordinates of 30.0000° S, 25.0000° E. With regard to the South African population, most people (36%) live in a temperate/ mesothermal climate with dry winters, followed by 34% of the population that live in a temperate/ mesothermal climate, with significant precipitation in all seasons, while 21% of the population live in a semi-arid climate. Only a small percentage (1.6%) of South Africans live in a semi-arid/ steppe climate, while 0.6% of the population live in a tropical wet and dry / savannah climate.[130]

In Johannesburg (coordinates: 26.2044° S, 28.0456° E) the mean sunlight hours range between 8 hours 18 minutes per day in April and 9 hours 12 minutes each day in September. There is an average of 3182 hours (72%) of sunlight per year (of a possible 4383); the remainder of daylight hours (28%) are likely cloudy or with shade, haze or low sun intensity. The average is 8 hours 42 minutes sunlight per day. At midday in Johannesburg the sun is on average 63.3° above the horizon.[132] As sunlight exposure is required for cutaneous vitamin D production, it is important to be cognisant of the sunlight hours, latitude, season and weather[2] of the region of the study. Johannesburg, South Africa, has more than adequate daily sunlight hours for vitamin D production.

In comparison, in Cape Town (coordinates 33.9253° S, 18.4239° E), the mean sunlight hours range from 6 hours 54 minutes per day in April to 7 hours 12 minutes per day in September. There is an average of 2993 (68%) hours of sunlight per year (of a possible 4383); the remaining 31.7% of daylight hours are likely cloudy or with shade, haze or low sun intensity. The average is 8 hours 11 minutes of sunlight per day. At midday in Cape Town the sun is on average 55.6° above the horizon.[94] Although Cape Town is situated further south of the equator than Johannesburg, it has enough sunlight hours. The vitamin D production in Cape Town during winter months might be lower due to the zenith angle of the sun. Cape Town sunlight hours were included as a matter of interest to demonstrate that the southern and northern parts of South Africa have very similar sunlight hours. Average daily temperatures in Johannesburg for the study period (April 2014 – September 2014) were 19ºC versus
17ºC for the same period in Cape Town.[122]

Sun exposure according to the calculated sun index was at its highest in the winter month of June 2014, where the average temperature in Johannesburg was 17ºC. Sun exposure was similar but lower than that of June during the months of May and July, when the average temperature in Johannesburg was 15ºC and 20ºC respectively. The colder winter months do not seem to influence sun exposure times. Participants might be outdoors for longer periods, but with less BSA exposed because of the colder weather. The lowest sun exposure was recorded in August; this might be due to the windy weather usually experienced during the month of August. Sun exposure then increased again during September, owing to the warmer weather (average 24ºC).[122]

Rosso et al. documented a statistically significant increase in risk of squamous cell carcinoma (SCC) when sun exposure exceeded the threshold of more than 70 000 accumulated hours of sun exposure in a lifetime.[115] This can be calculated in a 70-year-old individual as 1000 hours per year; thus 19 hours 10 minutes per week or 2 hours 44 minutes per day. Using the calculated sun index of this research project and assuming a sun exposure of arms (0.14 body surface area) and legs (0.24 body surface area), the sun index would be 19.18. This equates to very high sun exposure. Only one person in this study population had a calculated sun index above 7; this is much lower than the threshold for SCC risk. Basal cell carcinoma (BCC) showed a two-fold increase of risk for much lower sun exposure (8000–10 000 accumulated hours in a lifetime, thus 114–143 hours per year, thus 2 hours 12 minutes to 2 hours 44 minutes per week).[110] Using the calculated sun index of this research project and assuming a sun exposure of arms (0.14 body surface area) and legs (0.24 body surface area), the sun index would be 0.83–1.04 for BCC. The 75thpercentile of this study population has a calculated sun index value of 2.075. This is higher than the threshold for BCC risk; however, the median sun index for the group is lower than the BBC risk threshold. There is always the question of increased cancer risk when higher sun exposure is recommended. According to the data on total lifetime sun exposure and cancer risk, it would seem that this population with its current levels of sun exposure is not at higher risk of developing skin cancer. According to Amstrong et al., there is a strong correlation between skin cancer and early childhood sunburn episodes.[108] Childhood sun exposure was not recorded in this study population. Sun exposure guidelines and risk of skin cancer remain areas that need further investigation.

A novel approach to sun exposure was used in this study where time spent outdoors (hours per week) and body surface area exposed (percentage expressed as a decimal) were used as a calculated sun index. A similar method was used by Binkley et al., where skin colour was measured by reflectance colourimetry with a scale ranging from black (0) to white (100); added to this, a sun exposure index was used to determine amount and duration of skin sun exposure where subjects depicted their usual amount of skin exposed on a diagram. The rule of nines was used to calculate skin sun exposure.[15, 86] This number was then multiplied with reported weekly sun exposure hours.[15] The principles of this method were simplified and adapted for this study. Another method used in the study by Salamone et al. used a sunshine score that focused on time spent outdoors (little=1 and frequent=2) and the amount of sunlight exposure received (low=1 and high=2). A sunshine score, calculated as the sum of these two responses, was then classified as low (total =2) or high (total 3 or 4). A sunlight exposure score was added to this where a score between 0 and 9 was allocated according to time spent outdoors in last week, degree of skin exposure, sunblock use, and travelling.[33]

A calculated sun index (used in this study) value of 0.615 would express the clinical recommendation[2,16,31,35] for sun exposure. This would mean 30 minutes of sun exposure three times per week (total time 1.5 hours/week) on arms (0.14 body surface area) and legs (0.24 body surface area).This recommendation should be obtainable during lunch hours and/or weekends, as South Africa is well known for its sunny weather and outdoor lifestyle.

Pre-operatively the median calculated sun index for the study population was 0.715; thus 50% of the study population had higher sun exposure than the clinical recommendation. The sun index value of 0.715 can be interpreted as 38 minutes of sun exposure three times per week (total time 1.88 hours) on arms (0.14 body surface area) and legs (0.24 body surface area). The highest sun index recorded was 10.25, which indicates very high sun exposure. This means 3 hours 51 minutes of sun exposure seven times per week (total time 26.97 hours) on arms (0.14 body surface area) and legs (0.24 body surface area).

Post-operatively the median calculated sun index for the study population was also 0.715, indicating the same median sun exposure as pre-operatively. The highest sun index value recorded during the post-operative follow-up was 2.94; this translates to 2 hours 35 minutes of sun exposure three times a week (total 7.73 hours per week).

In closing: this study population had higher sun exposure than the clinical recommendation in the literature, and with a latitude of below 37º, Johannesburg is ideally situated to provide adequate sunshine, even in winter months. This amount of sun exposure is not indicated as enough sun exposure to be classified as increased risk of skin cancer.

4.3 Vitamin D Oral Intake

The guidelines for daily requirements for vitamin D intake from the Endocrine Society[37] (1500–2000 IU/day) are used as reference in this study as the study population can be considered an 'at risk' group as the participants of the study population were hospitalised for elective orthopaedic surgeries. The Institute of Medicine (IOM) adequate intake of vitamin D at 400–600 IU/day is recommended for a healthy population.[79]

Blalock et al. found that the mean differences between estimating vitamin D from a short screening food frequency questionnaire versus using a seven-day food diary was 47 IU/day. The positive predictive value of the short screening food frequency questionnaire in identifying persons with low vitamin D intakes was 100.0%.[117] Using the adapted short food questionnaire for vitamin D (Addendum G) that consists of the food available in South Africa with the highest vitamin D content (19 food choices), the average daily vitamin D intake was calculated. The average vitamin D intake from the individual food sources indicates that the most common source of vitamin D in the study population was eggs (average intake of 109.8 IU/day), followed by margarine (12.7 IU/day), butterfish (11.5 IU/day), health bars (11.4 IU/day) and FutureLife® cereal (10.5 IU/day). With these results of vitamin D intake from food sources, extra groups might be considered to add to the at-risk list in South Africa. These are individuals with egg allergy who avoid eggs, as well as vegetarians and specifically ovo-vegetarians, as the two main sources of vitamin D from food in this context are eggs and fish.

The median daily oral intake from food sources of the study population was 195 IU; this is only 13% of the lower range of the daily requirements of the Endocrine Society of 1500–2000 IU/day.[18,37] The 25th and 75th interquartile ranges of 142 IU and 266 IU respectively were also far below the recommended intake of the Endocrine Society. When vitamin D supplementation intake was added to oral intake, the median intake only increased to 202 IU/day; this is 13.5% of the lower range of the recommended intake of the Endocrine Society. It is clear that this population is not nearly attaining oral vitamin D intake in relation to these guidelines. When comparing the daily oral intake with the IOM guidelines (400–600 IU)[18] developed to meet the needs of at least 97.5% of the population according to age,[79] the intake is still only approximately 50% of the recommendation.

When comparing study data with the international findings of Wacker and Holick, the mean daily vitamin D intake from food and food plus dietary supplements from these two large surveys, the Continuing Survey of Food Intakes by Individuals (CSFII 1994–1996 and 1998) and the Third National Health and Nutrition Examination Survey (NHANES III USA 1988–1994), intake ranges from just more than 100 IU–350 IU for both studies.[18] These intake values are also far below (17%–58%) the recommendation of the IOM of 400–600 IU for adults and the Endocrine Society recommendation of 1500–2000 IU per day (6%–23%).[18,37] American oral intake data for vitamin D is slightly higher than the findings in this study from South Africa; these differences can be attributed to the fact that South Africa has very few food items fortified with vitamin D.[12]

It is very clear that the oral intake of vitamin D from food alone (as the body's sole source) will not be enough to reach adequate 25(OH)D. The response increment of 25(OH)D is 1–0.7ng/mL/100 IU.[18,34,84] With vitamin D intakes of around 200 IU, 25(OH)D contribution from oral intake is only around 2ng/mL.

A small proportion of the study population used supplementation (21%). The median intake was 400 IU/day which is 27% (only supplementation) of the lower range of the Endocrine Society daily requirement of 1500–2000 IU.[18,37] The maximum intake from supplementation was 1000 IU/day, only 67% of the daily requirements. The source of the supplementation was multivitamins (Pharmaton, Centrum and Biogen containing 400 IU vitamin D each); calcium supplements (Menacal7, BCalD and Caltrate containing 400 IU vitamin D each); Staminogro (containing 150 IU/tablet); and a pure vitamin D supplement (Solal) containing 500 IU/tablet. The majority of vitamin D came from combination

supplements like multivitamins and calcium supplementation. Vitamin D supplementation from pure vitamin D supplements was rare in this study group. At the time of data collection (April 2014 to November 2014), more than half of the vitamin D supplements available today were not yet on the market in South Africa. Most vitamin D supplements contained 500 IU or less. Table 1.6, the list of supplemental sources of vitamin D in South Africa, was updated in November 2016. The majority of vitamin D supplements containing more than 500 IU only became available recently in 2016 as supplements. The guidelines with regard to the quality, safety, dosage and efficacy requirements for the registration of health supplements are published by the Medicines Control Council (MCC) of South Africa. These guidelines have recently been updated. The allowable levels and claims for vitamins are contained in the MCC Complementary Medicines – Health Supplements Quality, Safety and Efficacy document, Version 2, published in June 2016. The maximum vitamin D dose that is allowed in an over-the-counter supplementation is 1 000 IU.[101] Vitamin D doses higher than 1 000 IU are available on prescription as 2 000 IU or 5 000 IU by Metagenics® (not scheduled) or 5 000 IU /ml oil or 50 000 IU tablet by Lennon (Schedule 3).

Deficiencies can be addressed by high dose supplementation of 50 000 IU once a week for eight weeks, followed by 50 000 IU every 2–4 weeks thereafter or 6000 IU daily for eight weeks followed by maintenance therapy of 1500–2000 IU/day as recommended by the Endocrine Society.[37,81] The risk of toxicity is very low, as no toxicity has been reported with supplementary doses of 10 000 IU or less per day.[35]

4.4 Vitamin D Status

In this study, vitamin D insufficiency was defined as vitamin D levels between 20 and 30ng/mL and vitamin D deficiency was defined as vitamin D levels below 20ng/mL.[2,3,6,8,9,14,16,18,37,50] There is no absolute consensus as to what the normal range for vitamin D should be; in global studies values of 20ng/mL are often indicated as sufficient and those below 20ng/mL as deficient.[18,24,132] Holick et al. reported that a dot plot graph of serum 25(OH)D as a function of PTH levels provides insight into what vitamin D levels should be. It was observed that the PTH levels began to plateau when 25(OH)D levels were between 30 and 40ng/mL.[3] Heaney and Holick measured intestinal calcium absorption in woman with 25(OH)D levels of 20ng/mL and 32ng/mL; they reported a 45–

65% increase in calcium absorption at the higher levels.[84] The preferred level for 25(OH)D is now recommended by many experts to be >30ng/mL.[3,8,18,84] With this in mind, deficiency was defined as <20ng/mL, insufficiency as 20–29ng/mL and sufficient as 30–100ng/mL for the purpose of this study. There have been numerous different guidelines used over the years to define vitamin D deficiency, ranging from < 5ng/mL[24] to 20ng/mL. These low ranges complicate interpretation of older data when deficiency is defined at such low values. A previous vitamin D classification of 'sufficient' might have to be reclassified as 'insufficient' or 'deficient'. It is thus recommended to always refer to the actual value of serum 25(OH)D when citing research.

Deficiency is highly prevalent in the Middle East (mean values of 12.8ng/mL in Turkish women), Asia (mean values of 14.4 in Chinese adolescent girls in Beijing), and India (mean values of 12ng/mL in hospital staff).[24] A moderate to poor25(OH)D is also commonly seen in Africa (9.4ng/mL in Ethiopian men and women; 17ng/mL in non-veiled woman in Tunisia),[9] in part owing to their dark skin types. In Europe, when examining 25(OH)D levels lower than 20ng/mL, deficiency ranges from 40–80% in the various adult age groups.[24] Vitamin D status appears to be much better in North America, where vitamin D deficiency is not so common, but vitamin D insufficiency (20–30ng/mL) is still common. Most studies are done on specific populations, such as the elderly, residents of institutions, the disabled, specific ethnic groups, populations with certain dress customs, and other high-risk groups.[18,24,27] Based on all these findings, it has been estimated that up to one billion people worldwide are vitamin D insufficient or deficient.

Pre-operatively the mean vitamin D status of this study population was classified as insufficient (26ng/mL, SD 9.6). The cumulative percentage of insufficient (35.8%) and deficient (29.9%) is 65.7% (<30ng/mL). There is little data available on orthopaedic patients in Africa and South Africa. In 1978, Pettifor et al. recorded mean serum 25(OH)D as 17.6ng/mL in South African patients with hip fractures: the population was an elderly population with a mean age 72,7± SD 13 years.[27] Coussens et al. recently reported values of <20ng/mL in 4–16%(summer) and 64–70%(winter) of participants in different ethnic groups respectively (Xhosa and Cape Mixed ancestry).[133] In a review done by Abhimanyu et al. on 25(OH)D and tuberculosis in South Africa, it is apparent that Asian-Indians are vitamin D deficient (16ng/mL), blacks are insufficient (28ng/mL), and whites are sufficient (34ng/mL). All values are mean values and deficiency, insufficiency, and sufficiency are defined as <20, 20-29 and 30-100ng/mL respectively.[134] Comparative global data show

Indonesians to be insufficient (23.6ng/mL), South Indians deficient, (8ng/mL), West Africans insufficient (27.6ng/mL), East Africans sufficient (46ng/mL), Dutch deficient (19.6ng/mL), Germans deficient (18.4ng/mL), French insufficient (20ng/mL) and British deficient (11.6ng/mL).[134] These differences are most likely due to a mixture of skin colour, clothing, sun exposure, vitamin D intake, climate and latitude, inter alia.

In a study done in Israel, the prevalence of hypovitaminosis D was 26.7%, with hypovitaminosis defined as <15ng/mL,[114] much lower than the current cut-off values for deficiency (<20ng/mL). This might explain why deficiency levels were lower in the Israeli study than in the current study. The population group in the Israeli study comprised patients admitted to an internal medical ward with no known risk factors for hypovitaminosis D.[114] The levels of vitamin D deficiency and insufficiency found in this current study were probably higher than expected because in Johannesburg, South Africa, there is abundant sunshine, even in the winter months. The findings might be in part explained by low vitamin intake from food, low supplementation use, low sun exposure, high BMI, and skin colour.

Post-operatively the mean 25(OH)D of this study population was insufficient (28.2ng/mL, SD 10.2). The cumulative percentage of insufficient (39.1%) and deficient (21.7%) was 60.8%. The difference between pre- and post-operative mean values is not significant. These levels of insufficiency might warrant supplementation or increased sun exposure. Supplementation is becoming more affordable as new supplements enter the market. Food fortification is a simple strategy that is also low cost, but great care should be taken in choosing the food vehicle and level of fortification to target the correct population groups. Consumer awareness of fortification is also important when targeting a specific population group.

When IOM vitamin D status guidelines are used the cumulative percentage of insufficiency and deficiency was 30%, and sufficient levels were 70%. The IOM states that practically all persons, that is 97.5% of the general population, are assured bone health when serum levels of 25(OH)D are >20 ng/ml. When the recommendation of >16ng/mL is used then 14% (n=9) of the study population have deficient vitamin D status. The IOM states that about half the general population are assured bone health when serum levels of 25(OH)D is >16 ng/ml.

International data available on post-surgery vitamin D status mostly relates to gastric bypass surgery and is thus not comparative data, as morbidly obese patients have been reported to present with vitamin D insufficiency.[103] In the current study, median values of 25(OH)D were 28ng/mL in April, then decreased in the first two months of winter (May and June), were higher in July, decreased in August, and then were higher again in September. In June and July the widest ranges of 25(OH)D levels were recorded: from >10ng/mL to <50ng/mL. The current study was conducted mostly over autumn, winter and early spring. Because the whole-body half-life of 25(OH)D from sun exposure can be as long as 6–8 weeks,[45] 25(OH)D status can still be quite high at the beginning of winter. It can also be speculated that vitamin D might be higher later in winter because winter daytime temperatures in Johannesburg are very pleasant and there is little to no cloud cover; outdoor activity can therefore still occur. August is known as the windy month in Johannesburg; outdoor activity might thus decline, and September sees the beginning of spring with higher temperatures and more outdoor activities.

4.5 Relationship between 25(OH)D and Parameters Pre- and Post-Operatively

Pre-operatively the correlation between age and 25(OH)D in this study population was not significant. Ageing is documented as a risk factor for vitamin D insufficiency and deficiency.[2,6,16,19,24] Some of the highest 25(OH)D levels were found in two subjects over 70 years of age (54ng/mL and 51.6ng/mL). Granted these two subjects also scored the highest with regard to their calculated sun index, so this was to be expected to some degree. Mean 25(OH)D was higher in males (29.2ng/mL) than in females (24.5ng/mL), although not significantly so (p=0.057). Post-operatively there was no correlation with 25(OH)D and age, probably because of the small sample size. Although the mean difference of 25(OH)D between males and females increased post-operatively from 4.7ng/mL to 5.8ng/mL, it was still not statistically significant (p=0.316). In another study[114] there was also no significant difference in 25(OH)D, with slightly higher levels in males than in females.

Pre-operatively non-smokers (27ng/mL) had slightly higher 25(OH)D levels than smokers (22.8ng/mL), but this did not reach significance (p=0.126). Post-operatively there was no statistically significant difference between smokers and non-smokers. It should be noted that the post-operative sample size was very small (n=23) and only two participants

smoked. Lange et al. also found no significant association between smoking and 25(OH)D levels (p=0.2). In the Lange study, smoking was significantly and inversely associated with lung function (p=0.0001). Vitamin D deficiency also had a negative effect on lung function, suggesting that vitamin D-deficient subjects that smoked the same number of cigarettes as vitamin D-sufficient subjects had lower lung function. This suggests that maintaining a sufficient vitamin D status may have a protective effect against the more rapid decline in lung function as seen in smokers.[135]

Skin classification with the Fitzpatrick Skin Phototype Classification was used in this study as in many others,[2,87-91] as it gives a more accurate description of the skin colour of the population than ethnic group classification alone, since people within different ethnic groups can have different skin tones. Pre-operatively vitamin D status according to skin type showed lowest mean 25(OH)D levels in skin type I (lightest) and skin type VI (darkest); however both these groups had only one subject so they were omitted from further analysis. Skin type III (white skin, burns minimally, tans well) had the highest mean 25(OH)D values (30.1ng/mL; SD 9.4). This skin type will produce more vitamin D because the skin is lighter and tans moderately and gradually. This group may be less likely to avoid sun exposure extensively. Skin type V (brown skin, rarely burns, tans deeply) differs significantly (p=0.025) from skin type III, as skin type V had the lowest25(OH)D mean level of the remaining skin types in the analysis. Thus the study findings are in line with the well-documented fact that skin pigmentation (darker skin) absorbs UVB radiation via melanin and thus produces less vitamin D.[2,6,8,16,19,24,35] In the post-operative group (n=23) there was no difference (p=0.389) between the different skin types, probably owing to the small sample size. In this group, skin type II (white skin, always burns, minimal tan) had the highest mean 25(OH)D level (31.8ng/mL) and skin type V still had the lowest 25(OH)DD level (21.5ng/mL).

Obesity is a known risk factor for vitamin D insufficiency and deficiency as an increased BMI is related to decreased vitamin D status because vitamin D is sequestrated in fat stores.[2,6,16,19,24,35] Fat stores can be an irreversible sink for vitamin D in obese individuals. Holick observed that obese subjects could exhibit increases in blood vitamin D concentrations of less than 50%, when compared with non-obese individuals, after supplementation.[2] The mean BMI of this study population was 31.33kg/m^2 (Obese Class I); however there was no correlation with 25(OH)D and BMI (pre-operatively). No correlation was found post-operatively between anthropometric measurements and vitamin D status.

A study conducted in bariatric surgery patients (Roux-en-Y gastric bypass surgery) [RYGBP]) investigating vitamin D status, found that more than 90% of the population screened had some sort of hypovitaminosis D (25.0% with insufficiency, 68.8% with deficiency). The average BMI of the bariatric group was >48kg/m^2 (Obese Class III),[115] compared with 31.3kg/m^2 of this study group. It is not clear at what level of BMI vitamin D insufficiency is more likely to occur.

Pre-operatively a positive correlation (p<0.001) between calculated sun exposure index and 25(OH)D was found. Calculated sun index was a weak predictor of vitamin D status in this population group (R^2=0.103). While there was a positive correlation pre-operatively, there was no correlation post-operatively between 25(OH)D and calculated sun exposure index. Again, this might be due to the small sample size of this group. It should be noted that as shown in previous research studies, high sun exposure does not necessarily guarantee sufficient vitamin D status (>30ng/mL). In the cohort of young adults in the Binkley et al. study, there was considerable variability in vitamin D concentrations even though abundant sun exposure was documented.[17] Additionally, despite the amount of sun exposure, the 25(OH)D concentration did not exceed approximately 60ng/mL.[15] In this regard it is acknowledged that human skin has the intrinsic capability to limit vitamin D production.[2] It is definitely plausible that genetic differences are present in the quantity of vitamin D needed to continue optimal physiological function. Thus, it is probable that factors may exist which we do not yet fully understand that can limit skin production of vitamin D in response to UV exposure. It is important that we do not wantonly agree to the notion that vitamin D deficiency and insufficiency are solely due to too little UV exposure.[15] It seems self-evident from this and other studies that insufficient vitamin D status, as it is currently defined in the literature, could occur regardless of “more than adequate” sun exposure.[2,15,86] Sun exposure in this and other studies took into consideration the use of sunscreen and body surface exposed to sunlight. The sun exposure calculated was without sunscreen, and protective clothing was taken into consideration.[86]

The response increment for 25(OH)D is 1–0.7ng/100 IU.[18,34,84] Pre-operatively the median vitamin D intake from food ranged from 24 IU/day to 511 IU/day. It seems very unlikely that vitamin D status can be maintained from food intake alone in this group. The response from vitamin D intake on 25(OH)D will only be 0.2ng/mL to 5.1ng/mL. Thus it is understandable that there is no correlation between 25(OH)D and oral intake in this study as intake was too low. When adding supplementation intake to the equation, the minimum

intake ranged from 24–1227 IU/day. The highest response to 25(OH)D from total intake will only be 12.3ng/mL, still deficient in meeting vitamin D sufficiency of 30ng/mL. Post-operatively, vitamin D intake from food increases marginally from 67–669 IU per day. No correlation between 25(OH)D and oral intake in the post-operative group was noted. When adding supplementation intake to the equation, the intake was 67–1215 IU per day. The highest response to 25(OH)D from total intake will only be 12.2ng/mL, still not adequate in meeting vitamin D sufficiency of 30ng/mL.

4.6 Pre-operative Parameters in Relation to Post-Operative Parameters

One of the aims of this study was to determine if the orthopaedic surgical intervention would change sun exposure habits and thus impact on vitamin D status. It was found that the calculated sun index (thus sun exposure time and body surface area (BSA) exposed) did not change significantly from the pre-operative to post-operative period. It can be assumed that with major orthopaedic interventions such as hip replacement and back surgery, where the recovery period would be much longer and sometimes even include rehabilitation, there would be an influence on sun exposure habits after surgery as well as before surgery as patients might be immobile for extended periods of time. In this study population there were only 12% major surgeries included, and 88% minor surgeries that could have had an impact on the mobility of patients. The null hypothesis is thus retained that there is no statistically significant change in sun exposure after orthopaedic surgical impact compared with the pre-surgery sun exposure. In other major orthopaedic surgeries this might not be the case, as seen in patients with lumbar spinal stenosis where vitamin D status improved significantly after surgery (one year later) (p=0.017).[136] In this study the short-term impact of the surgical intervention was not investigated. The authors hypothesised that decompression surgery improves physical function in patients with lumbar spinal stenosis, and the resulting improved physical function will increase serum vitamin D levels by preventing hypovitaminosis D resulting from high bone turnover caused by restricted mobility, increased sun exposure from improved walking ability, and subsequent improvement in nutritional status.[136] In this study the long-term benefits were not explored as follow-up period was 78 days (SD=25).

In this study it has already been established that vitamin D intake from food and supplementation does not significantly influence 25(OH)D pre- and post-operatively. According to the data collected, the participants did not substantially increase their vitamin D food intake or supplementation intake post-operatively. The null hypothesis is thus retained that there is no statistically significant change in vitamin D intake after orthopaedic surgical impact compared with that prior to surgery.

There was a very small mean paired difference in 25(OH)D between the pre-operative and post-operative group of 0.5ng/mL ± SD=5.03. It could be hypothesised that the orthopaedic surgical impact and accompanying recovery period might impact 25(OH)D negatively. This might be the case in major surgeries like hip replacements and back surgeries where mobility is impaired in the short term during recovery; however in the long term the reverse might also be true, where 25(OH)D improved post-operatively owing to better mobility.[136] In this study population the null hypothesis is retained that there is no significant change in 25(OH)D after orthopaedic surgical impact and recovery compared with the pre-surgery status. This study could be repeated in a study population consisting of only major surgeries to determine if surgical intervention will impact the vitamin D status in the short and long term.

The calculated sun index for the clinical recommendation[16,31,35] of sun exposure to ensure adequate vitamin D as recorded in the literature equates to 0.615. In this study population (pre-operatively), 80.6% of the population with sun exposure less than the clinical recommendation was insufficient and deficient in vitamin D status. It is very important to also note that in the sub-group that had enough sun exposure according to the clinical recommendation (>0.615), only 47% had sufficient vitamin D. Just over half (53%) of the group with enough sun exposure according to the clinical recommendation still had insufficient and deficient vitamin D serum levels. Similar results were found in the post-operative group where 78% of the group without enough sun exposure had insufficient and deficient vitamin D status and half of the participants had insufficient and deficient vitamin D status even with enough sun exposure (according to the clinical recommendation in the literature).Thus, even ample sunlight exposure does not guarantee adequate 25(OH)D for all persons, according to the currently accepted clinical recommendations. This implies that in this study and in the study of Binkley et al., the current clinical recommendation to allow sunlight exposure to the arms and legs (not face) and without sunblock for 15–30 minutes two to three times a week between 11:00 and 15:00 in spring, summer and

autumn[16,31,35] may possibly not ensure the production and synthesis of sufficient vitamin D.[15] Dietary intake of vitamin D was not included in the Binkley study.

4.7 Predicted Calculated Sun Index Levels for Sufficient Vitamin D Status

When the ROC curve is used to predict a more favourable outcome for 25(OH)D and recommended sunlight exposure, a sensitivity of 68.2% can be achieved versus 53% with a calculated sun index of 1.159. The specificity will be 56.5%. If the same BSA exposure is used as in the clinical recommendation of the literature (arms and legs), the sun exposure time will double from 30 minutes to one hour. A calculated recommendation will then be 60 minutes sun exposure to arms and legs three times per week, or 30 minutes six times per week on the arms and legs. This is double the current recommendation. Even then sufficient vitamin D status cannot be guaranteed as it is known that there are many other factors that influence 25(OH)D, such as age, skin tone, BMI, season, and latitude, inter alia.

The fact that just more than a third of the participants in this study had sufficient levels of vitamin D is a worrying factor when it is taken into consideration that South Africa has abundant sunshine and that the median sun exposure of this study group does meet clinical recommendations. Oral intake of vitamin D is found to be very low and supplementation in this group is underutilised. This study group can be seen as an at risk group, as the mean BMI of the group is more than 30kg/m^2 (obese) and all participants were scheduled for elective orthopaedic surgery. As the function of vitamin D in bone health is well documented, greater care should be taken in ensuring optimal vitamin D status in this population group.

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

5 CONCLUSION AND RECOMMENDATIONS

5.1 Findings and Conclusions

Vitamin D is well documented to be essential in guaranteeing skeletal health, and as new research emerges it is clear that it plays an important role in overall health. What is truly remarkable is that in this modern day and age, with advances in medicine, vitamin D deficiency has resurged in all population groups. The question also might be asked if we are only recognising the problem now? Vitamin D insufficiency can be very subtle and for this reason can go unrecognised by health professionals.

This study aimed to shed some light on the current vitamin D status and factors influencing it in an orthopaedic surgical population in a private hospital in Johannesburg. In the current study sun exposure was slightly higher than the clinical recommendation; however this was not enough to guarantee adequate 25(OH)D levels (a third of the study population had insufficient levels and a third had deficient vitamin D levels). These high levels of vitamin D insufficiency were not expected in a country with such abundant sunshine all year round. These findings were relevant for light- and dark-skinned individuals. Oral intake of vitamin D from food and supplementation was also inadequate in terms of recommended daily intake. Not even a quarter of the current recommendations were met, as very few foods contain vitamin D and food fortification and supplementation is not common in South Africa. These parameters did not change significantly post operatively, thus it can be concluded that minor orthopaedic surgery will probably not influence pre-operative vitamin D status and other habits of sun exposure and vitamin D oral intake to any extent. Recommended sun exposure to predict more favourable outcomes would be at least double the current clinical recommendation.

In the current study age, smoking and BMI were not strong predictors of vitamin D status. Calculated sun index (sun exposure) was a moderately strong predictor of 25(OH)D. Subjects with a white skin that burns minimally but tans well had the highest 25(OH)D versus their darker counterparts with brown skin. Minor orthopaedic surgical interventions do not negatively influence patients' sun exposure habits, vitamin D intake, and thus vitamin D status, but these patients seem to start off with lower than expected 25(OH)D levels and might be considered to be an "at risk" group as their mean BMI classification is obese and elective surgery is scheduled.

Standard vitamin D testing is still not recommended in the general population, with testing only warranted in at-risk groups. Current studies propose that more vitamin D may be needed than presently recommended. Where more sun exposure is not possible or is contra-indicated, vitamin D supplementation of 50 000 IU per week for eight weeks followed by 50 000 IU every two to four weeks thereafter or 6 000 IU daily for eight weeks followed by maintenance therapy of 1500–2000 IU/day as recommended by the Endocrine Society is advocated.[37] This is a cost-effective and practical recommendation.

This study clearly highlights the need for more specific guidelines to obtain optimal vitamin D status. These guidelines need to distinguish between different groups in terms of skin tone, sun exposure habits and at-risk groups, where different sun exposure guidelines might be needed. Skin protection against skin cancer should be kept in mind. Education regarding food sources rich in vitamin D should also be included in the multi-pronged approach. If these guidelines cannot be met, supplementation should be considered. This study indicates that the orthopaedic surgical population is an at-risk group that can benefit from a multi-pronged approach to improve vitamin D status.

In this study, the null hypothesis is rejected where the hypothesis states that there is no statistically significant correlation between vitamin D status and age, vitamin D status and sun exposure, and vitamin D status and skin tone. The null hypothesis is retained where the hypothesis states that there is no statistically significant correlation between vitamin D status and gender, smoking, BMI, waist circumference, vitamin D intake and vitamin D supplementation. The null hypotheses were also retained where there was no statistically significant change in sun exposure, vitamin D intake, vitamin D supplementation, and vitamin D status after the orthopaedic surgical intervention.

5.2 Recommendations for Future Studies

Given the key roles that vitamin D plays in fractures, falls and orthopaedic pathways, this field of study deserves further attention. Testing and validating of the calculated sun index formulae tool could be suggested as a measure of sun exposure as the tool used in this study was a novel approach. There is also room for randomised control studies to determine the benefits of vitamin D supplementation in improving recovery after orthopaedic surgical interventions. Vitamin D supplementation, nutrition education, and

sun exposure education could be added as an intervention to improve vitamin D status and subsequently recovery from surgery. The study could be repeated with a study population including major orthopaedic surgical procedures such as hip replacements and back surgery where recovery periods are longer and sun exposure might be limited.

In a diverse society such as South Africa, there is a need to produce credible data on the vitamin D status of the population and the needs of individuals with varying skin tones. The various stakeholders should work in partnership to achieve these goals.

5.3 Study Limitations

Study limitations included the small sample size, large percentage of minor surgical interventions and the low number of patients retained in the follow-up phase that limited the data on post-operative vitamin D status, intake and sun exposure. In future the study population can be increased to ensure equal representation between skin tone variation groups and more major surgical interventions. Follow-up period can be adjusted for short and long term impact of orthopaedic surgery.

5.4 Declaration of Interest

No declaration of interest. There were no conflicts of interest in this study.

5.5 Deviations from Protocol

In this private hospital setting, surgical follow-up was between one and two weeks, and this period was too short for the vitamin D status follow-up to be conducted. The follow-up period was extended from a month to 7–17 weeks in a bid to increase follow-up numbers.

5.6 Sources of Support

None.

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Addendum A: Consent Form

PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

TITLE OF THE RESEARCH PROJECT:

Vitamin D status among surgical orthopaedic patients in a private hospital in Johannesburg, South Africa

PARTICIPANT NUMBER:

PRINCIPAL INVESTIGATOR: Ronelle Vermaak (Dietician)

ADDRESS:

Mulbarton Hospital

25 True North Road

Mulbarton

Medical Centre, Room 207

CONTACT NUMBERS:

011 432 3866

083 463 1730

You are invited to take part in a research project. Please take some time to read the information presented here, which will explain the details of this project. Please ask the principal investigator, Ronelle Vermaak, any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the **Health Research Ethics Committee at Stellenbosch University and the Netcare Research Committee** and will be conducted according to the ethical guidelines and principles of the international Declaration of

Helsinki, South African Guidelines for Good Clinical Practice, and the Medical Research Council (MRC) Ethical Guidelines for Research.

What is this research study all about?

The study will be conducted at Netcare Mulbarton Hospital, Johannesburg. A total of 87 participants will take part in the study.

In recent years, vitamin D has become a very hot topic in the research field. A recent study done in Israel (a sunny country) revealed vitamin D deficiency to be common. Vitamin D status has been done and studied globally in great detail. Studies on vitamin D status in Africa are few and far between, and most studies have been done on children.

This led to the idea that vitamin D status needed to be investigated in South Africa. We assume that our vitamin D status is efficient because we live in a sunny country. We do not know if the recommendations of the Cancer Association (sunblock use, protective clothing) and our changes in lifestyle have had an impact on our vitamin D status.

Associations can also be made between vitamin D status and other variables like weight, skin tone, sun exposure and food intake.

In this study you will be asked questions about your food intake, time spent in the sun and sunblock use. Your age, sex, weight, waist circumference and skin tone will be recorded. All interviews and measurements will be done in private. A blood sample will be taken by Lancet Laboratories to measure your levels of vitamin D before your surgery and a follow-up blood test will be done on your vitamin D levels a month after your surgery. Your food intake and sun exposure will be recorded again a month after surgery at the follow-up visit.

Why have you been invited to participate?

All patients that have elective orthopaedic surgery booked at Mulbarton Hospital during the period April 2014 – September 2014 (pending on ethical approval) will be asked to participate in the study.

What will your responsibilities be?

You will not be asked to take any medication or supplements other than what your doctor prescribes. The responsibilities of participants in the research project will be to be truthful and honest when answering the questions about food intake, sun exposure, skin type and

personal information. Participants will be weighed and measured (height and waist circumference), and asked for a blood sample (to test vitamin D status) before surgery. Participants will be asked to attend a follow-up visit one month after surgery where another blood sample will be taken for vitamin D status and questions answered about food intake and sun exposure.

Will you benefit from taking part in this research?

There are no personal benefits from this study, but you will be part of a selected group of patients that will contribute to a study that will be the first of its kind done in South Africa. With these results it will be possible to determine if orthopaedic patients have vitamin D deficiency or are at risk of becoming vitamin D deficient after surgery and if vitamin D supplementation is needed in future.

Are there in risks involved in your taking part in this research?

There are minimal risks involved in participating in the study. The medical staff from Lancet Laboratories will take the utmost care to minimise any discomfort involved in taking blood samples.

If you do not agree to take part, what alternatives do you have?

There will be no consequences if you decide not to participate in the study.

Who will have access to your medical records?

Only the investigator (dietician), supervisors and statistician will have access to the information gathered during the study. All the information collected will be treated as confidential and protected. If used in a publication or thesis, the identity of the participants will remain anonymous.

What will happen in the unlikely event of some form of injury occurring as a direct result of your taking part in this research study?

In the event of injury occurring as a direct result of the research, the investigator has the appropriate insurance cover as specified by Netcare Hospitals.

Will you be paid to take part in this study and are there any costs involved?

No, you will not be paid to take part in the study but the cost of all vitamin D blood tests will be covered by the researcher. There will be no costs involved for you if you do take part. If you incur any travel expenses to attend the follow-up visit, you will be reimbursed with a travel voucher to the maximum of R50.

Is there anything else that you should know or do?

- Your orthopaedic surgeon will be informed that you are taking part in the research study.
- You can contact Ronelle Vermaak, the dietician, at 083 463 1730 if you have any further queries or encounter any problems.
- You can contact the Health Research Ethics Committee at 021 938 9207 if you have any concerns or complaints that have not been adequately addressed by your study dietician.
- You will receive a copy of this information and consent form for your own records.

Declaration by participant

By signing below, I ……..……. agree to take part in a research study entitled **Vitamin D status among surgical orthopaedic patients in a private hospital in Johannesburg, South Africa**

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- I may be asked to leave the study before it has finished, if the researcher feels it is in my best interests, or if I do not follow the study plan agreed to.

Signed at (*place*) .....…..…….. on (*date*) ……...…….. 2013.

....

Signature of participant **Signature of witness**

Declaration by investigator

I *(name)* ……..…… declare that:

- I explained the information in this document to ……..
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above.
- I did/did not use an interpreter. (*If an interpreter is used then the interpreter must sign the declaration below.)*

Signed at (*place*) .....…..…….. on (*date*) ……...…….. 2013.

....

Signature of investigator **Signature of witness**

Addendum B: Anthropometric Measurements & Biochemical Data

Participant Number	

Date	D	M	Y
1. Weight		1	kg
		2	kg
		3	kg
		Average	kg
2. Height		1	cm
		2	cm
		3	cm
		Average	cm
3. Waist Circumference			cm
		Male:	
		<102cm	1
		≥ 102cm	2
		Female	
		<88cm	1
		≥88cm	2

4. BMI		kg/m²
	Underweight :<18.50	1
	Normal :18.50–24.99	2
	Overweight: 25.00–29.99	3
	Obese Class I: 30.00–34.99	4
	Obese Class II: 35.00–39.99	5
	Obese Class III: ≥40	6
5. 25-hydroxyvitamin D [25(OH)D]		ng/ml
	Undetectable: <5ng/ml	1
First Reading (Pre-Operative): Date:	Deficiency: 20ng/ml	2
	Insufficiency: <30ng/ml	3
	Optimum: >30ng/ml	4
	Toxic >100ng/ml	5
8. 25-hydroxyvitamin D [25(OH)D]		ng/ml
	Undetectable: <5ng/ml	1
Second Reading (Post-Operative): Date:	Deficiency: 20ng/ml	2
	Insufficiency: <30ng/ml	3
	Optimum: >30ng/ml	4
	Toxic >100ng/ml	5

Addendum C: Demographic Questionnaire

Participant Number:			
Date	D	M	Y
1. Type of Surgery	Hip Replacement		1
	Knee Replacement		2
	Shoulder		3
	Back		4
	Specify Other:		5
2. Gender		Male	1
		Female	2
3. Age			
4. Date of Birth (for control purposes, do not code)	D	M	Y
5. First Language	English		1
	Afrikaans		2
	Portuguese		3
	isiZulu		4
	isiXhosa		5
	Other		6
6. Second Language	English		1
	Afrikaans		2
	Portuguese		3
	isiZulu		4
	isiXhosa		5
	Other		6
7. Ethnic Group	White		1
	Indian		2
	Black		3
	Coloured		4
	Asian		5
	Other		6
8. Do you have any chronic disease?		Yes	1
		No	2

9. If yes, state disease.	Diabetes	1
	Cholesterol	2
	Hypertension	3
	Gout	4
	Specify Other:	5
10. Do you take any medication for your condition?	Yes	1
	No	2
11. If yes, state medication.		
12. Do you use any other medication?	Yes	1
	No	2
13. If yes, state medication and doses.		
14. Do you take any supplements?	Yes	1
	No	2
15. If yes, state supplements, doses and frequency.		
16. Do you smoke?	Yes	1
	No	2
17. If no, have you smoked regularly before?	Yes	1
	No	2
18. If yes, how much do you smoke?	Per day	
	Per week	
19. How long have you been smoking?	Years	

Addendum D: Skin Type Classification (The Fitzpatrick Skin Phototype Classification)

Participant Number:			
Date	D	M	Y

The Fitzpatrick scale	
Type FZ I: White skin, always burns, never tans	1
Type FZ II: White skin, always burns, minimal tan	2
Type FZ III: White skin, burns minimally, tans moderately and gradually	3
Type FZ IV: Light brown skin, burns minimally, tans well	4
Type FZ V: Brown skin, rarely burns, tans deeply	5
Type FZ VI: Dark brown/black skin, never burns, tans deeply	6

Addendum E: Sun Exposure

Participant Number:			
Date	D	M	Y
Pre-Operative			
Post-Operative			

Time spent outdoors between sunrise and sunset	**With sunblock**	**Without sunblock**	**Office use**
Weekdays			
< 15 minutes / day			1
15 – 30 minutes / day			2
30 – 60 minutes / day			3
1 – 2 hours / day			4
>2 hours / day			5
Weekends			
< 15 minutes / day			6
15 – 30 minutes / day			7
30 – 60 minutes / day			8
1 – 2 hours / day			9
>2 hours / day			10
Calculated hours per week			

Usual attire during sun exposure. Choose one from every category:

Clothing during the week						
Category 1			Category 2		Category 3	
No shirt	Long-sleeved shirt	Short-sleeved shirt	Short pants	Long pants	No hat	Hat

Clothing during the weekend						
Category 1			Category 2		Category 3	
No shirt	Long-sleeved shirt	Short-sleeved shirt	Short pants	Long pants	No hat	Hat

Office use only:

Week:

Body Area	Category 1			Category 2		Category 3	
	No shirt	Long-sleeved shirt	Short-sleeved shirt	Short pants	Long pants	No hat	Hat
	0.36	0.04	0.14	0.24	0.00	0.07	0.03

Weekend:

Body Area	Category 1			Category 2		Category 3	
	No shirt	Long-sleeved shirt	Short-sleeved shirt	Short pants	Long pants	No hat	Hat
	0.36	0.04	0.14	0.24	0.00	0.07	0.03

Sun exposure hours:		BSA exposed		Sun Index
Week		Week		
Weekend		Weekend		
Weekly		Weekly		

Sun exposure X BSA = Sun Index

Addendum F: Short Food Questionnaire for Vitamin D

This form asks about your *usual* eating habits over the past month (dietician will fill in form)

- For each food listed, mark the column to show how often, on average, you ate the food during the three months.
- Please BE CAREFUL which column you put your answer in.
- Mark whether your usual serving size is small, medium, or large. Please DO NOT OMIT serving size.

- Please DO NOT SKIP any foods. If you never eat a food, mark “Never or less that once a month”.
- A small serving is one-half or less of the medium serving size shown.
- A large serving is about one-and-a-half times or more of the medium serving size shown.

Participant Number			
Date	D	M	Y
Pre-operative			
Post-operative			

											HOW MUCH				OFFICE USE ONLY		
Type of Food	Never or Less than Once Per Month	1 Per Month	2-3 Per Month	1 Per Week	2 Per Week	3-4 Per Week	5-6 Per Week	1 Per Day	2+ Per Day	Medium Serving	**S**	**M**	**L**	**FOOD CODE**	**g Intake/day**	**IU/ day**
Cod liver oil														1		
Salmon, canned, drained														2		
Sardines, Canned in oil, drained														3		
Sardines, canned in tomato sauce														4		
Herring, grilled														5		
Kipper, baked (smoked herring)														6		
Butterfish, grilled														7		
Pilchards, canned in brine / tomato sauce														8		
Tuna, canned in oil, drained														9		
Egg, whole														10		
Liver, beef														11		
Liver, chicken														12		
Pork														13		
Beef														14		
Veal														15		
Cereal: Specify														16		
Cereal: Specify														17		
Margarine: Specify														18		
Milk Powder:														19		
Health Bar: Specify														20		
Health Bar: Specify														21		
														TOTAL		

Addendum G: Lancet Laboratories venepuncture procedure[118]

Activity	Procedure	Reason
Preparation		
Greet Patient	• Friendly	• Good manners • The patient was made to feel welcome.
Identify Patient	• All in and out of hospital patients were identified	• To ensure blood was being collected from the correct patient.
Ensured patient preparation & clinical information	• Checked if the patient was fasting. • Checked if any information about medication needed to be recorded. • Made sure that the patient had signed consent for the tests being done.	• Requirement for specimen collection. • Some results might be interpreted according to medication of the patient.
Explained the procedure to the patient	• Asked patient if he/she had had blood collected before; if not, briefly explained the procedure.	• Ensured that the patient was aware of what was happening to them and allayed anxiety.
Selected & prepared equipment	• BD Vacutainer needle 21Gx1.5 • Vacutainer holder tourniquet • Gloves • Alcohol swab • Cotton wool swab • Plaster	• Was efficient and made sure that everything was ready,
Chose correct containers/tubes	• One SST tube (gold/rust) • 5ml of blood drawn	• For vitamin D test.
Checked expiry date on tubes	• Checked tube expiry dates	• Accuracy of test results.
Washed hands or sprayed	• Used the Lancet hand-washing procedure.	• Prevented the spread of infection.
Latex allergy / Applied gloves	• Gloves were worn for all procedures • Asked the patients if they were allergic to latex; if allergic to latex used Nitrile gloves (blue)	• To protect sister and the patient from biohazards.

Activity	Procedure	Reason
Positioned the patient	• Sat or laid the patient down comfortably and in safe position. • Extended the patient's arm in a downward position; made sure that the arm was supported.	• Ensured that the patient was comfortable and safe.
Applied the tourniquet	• Applied the tourniquet 10cm above the intended puncture site, tightened. • Did not leave the tourniquet tightened around the patient's arm for longer than one minute.	• The tourniquet caused the veins to bulge and made them prominent.
Selected site	• Asked the patient to close the fist. • Chose a suitable site and palpated the vein.	• Ensured that the specimen was collected from a suitable site.
Cleaned site	• Used an alcohol swab, cleaned the selected site in a circular motion.	• Ensured clean procedure.
Showed patient sealed needle	• Told the patient that the needle was sealed and showed it to him/her.	• Gave the patient confidence that the needle was a new one.
Blood Collection		
Inserted needle/ Didn't bend	• Performed the venepuncture procedure.	• The needle was not be bent.
Advanced tubes onto the vacutainer assembly	• Made sure that the vacutainer holder was kept steady while attaching the tubes.	• Filled the tubes with blood. • 5ml of blood was collected.
Released the tourniquet	• Once blood flow was established, the tourniquet was loosened.	• Ensured accurate results. • Prevented bruising.
Mixing of tubes / Inverting	Mixed the tubes by inverting them gently 5–6 times	• Mixed the additive in the tube with the blood, promoted clotting of blood.
Removed needle and applied pressure	• The blood flow was stopped with a cotton swab. • The needle was removed and was discarded in the sharps bin immediately.	• Stopped the flow of blood and prevented a haematoma from forming.

Activity	Procedure	Reason
Asked about plaster allergy / Applied plaster	• Asked about plaster allergy before applying plaster.	• Kept the venepuncture site covered.
Completion		
Disposal of waste material	• Ensured that material was disposed of in the correct waste container. Only material that was in contact with the patient and the patient's body fluids was disposed of in the biohazard bin.	• Prevented unnecessary exposure to bio-hazardous materials.
Labelled tubes: **Patient details**	• Wrote down the patient's surname, initials and date of birth or identity number as well as the gender of the patient, i.e. M/F.	• Ensured that the specimen did not get muddled up with another patient's specimen.
Barcoded tubes& form	• Placed barcode straight as close to the top of the tube as possible.	• Ease of scanning and processing throughout the laboratory system was ensured.
Packaged tubes	• Tubes were put in the clear side of the specimen bag, so that they could be seen and closed the Ziploc section.	• For safe secure transportation of the specimen.
Washed hands	• As per the Lancet hand-washing protocol.	• Prevented cross-infection between patients and ensured personal hygiene.
Transported specimens	• Specimens were packed in a cooler for transport. • Specimens were send to Lancet main laboratory (Richmond, Johannesburg).	• Results were available within 48 hours. • Specimen were kept for 7 days if further test were required